SLEEPING LIKE A BABY: SLEEP AND GUT BACTERIA IN DEVELOPMENT

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Sleeping like a Baby: Sleep and Gut Bacteria in Development

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A baby will make love stronger, days shorter, nights longer, bankroll smaller, a home happier, clothes shabbier, the past forgotten and the future worth living for.

— Anonymous

ABSTRACT

Human development is a complex interplay of different factors. This thesis focuses on two of these factors: sleep and gut bacteria. Sleep takes up more than half of each day in infancy and childhood, and gut bacteria make up around half of all cells in our body. Both undergo large transitions in infancy and childhood. Sleep has been suggested to be a marker of brain maturation. Gut bacteria have been suggested to be health markers. Studies in rodents and adults have found the first evidence that sleep and gut bacteria are linked. However, no studies have examined this link in infancy - a phase with rapid developmental changes.

This thesis fulfills two aims, which are addressed in 6 research articles. The first aim is to get more precise and standardized estimates of infant sleep behavior by improving the methodology of infant actigraphy sleep research. The second is to characterize sleep across development, focusing on the link between sleep and gut bacteria.

The first aim was addressed in three articles. Article 1 systematically reviewed the existing literature that uses actigraphy to measure sleep in infants and children. We rated the methodological reporting, highlighting which information is currently underreported. Additionally, we made recommendations for future studies. Article 2 compared the estimates from two commonly used algorithms (Sadeh and Oakley/Respironics) that score sleep-wake patterns from movement. We found major differences between the sleep estimates but proposed a standardized pipeline to reduce these differences. In Article 3, we applied a principal component approach to a large array of sleep variables to find underlying sleep composites. We found 5 sleep composites, which accurately reflect sleep maturation in the first year of life: *Sleep Day* (measuring daytime sleep duration and regularity), *Sleep Night* (measuring nighttime sleep duration), *Sleep Timing* (measuring nighttime sleep timing), *Sleep Activity* (measuring awakenings and activity during the night) and *Sleep Variability* (measuring variability of night sleep timing and duration across measurement days).

For the second aim, we looked at sleep development across infancy, childhood, and adolescence. Articles 3 and 4 used a large cohort of 162 infants, measured at 3, 6, and 12 months of age with behavioral follow-up at 24 months. In Article 3, we characterized infant sleep in the first year of life. We confirmed the high variability between infants but also show a high variability within infants across the first year of life. We found stable sex differences in *Sleep Activity* already in infancy. Furthermore, we found that *Sleep Day* is associated with behavioral development. In Article 4, we investigated which role gut bacteria play in these associations. We showed that alpha diversity was associated with *Sleep Day*, especially in early development. Additionally, *Sleep Activity* was associated with bacterial maturity and enterotype. Furthermore, we found exciting associations between both sleep and gut bacteria and development; most notably, we extended the finding of *Sleep Day* as a maturational marker by showing that daytime sleep at 12 months negatively predicts gross motor development at 24 months. Additionally, we found a positive association between alpha diversity and early behavioral development. Overall, we showed complex and dynamic associations between sleep, gut bacteria, and behavioral development.

Articles 5 and 6 examined the spatio-temporal properties of sleep slow waves. In Article 5, we investigated a dataset of 29 children and adolescents between 2 and 17 years of age. We examined how sleep slow waves travel across the cortex, how this traveling behavior changes across the night, and if the traveling behavior is different across development. We found one aspect that had age-dependent changes across the night: slow wave propagation distance. It only decreases across the night in children younger than 5 years old. In Article 6, we reviewed the relationship of intracellular slow oscillations and scalp slow waves. Additionally, we showed that the origin of sleep slow waves is less likely to be located in frontal areas in children than in adolescents. We proposed that slow waves not only mirror brain maturation but might also be driving this maturation.

This thesis shows the remarkable development of sleep from infancy to adolescence. It also provides the first evidence of a sleep-gut link in infancy and reveals interesting associations between sleep and gut bacteria and behavioral development. I propose methodological recommendations and future research directions to increase our knowledge of infant development.

ZUSAMMENFASSUNG

Die menschliche Entwicklung ist ein komplexes Zusammenspiel von verschiedenen Faktoren. Diese Dissertation fokusiert auf zwei dieser Faktoren: Schlaf und Darmbakterien. Schlaf nimmt in der Kindheit mehr als die Hälfte des Tages in Anspruch, und die Darmbakterien machen die Hälfte aller Zellen im menschlichen Körper aus. Beide durchlaufen beträchtliche Transformationen in den ersten Lebensjahren. Schlaf wurde als Marker der Hirnreifung vorgeschlagen. Darmbakterien sind potentielle Gesundheitsmarker. Studien in Nagetieren und Erwachsenen haben erste Hinweise auf eine Verbindung zwischen Schlaf und Darmbakterien gefunden. Dies wurde bisher aber noch nie im Säuglingsalter untersucht - einer Phase mit markanten Veränderungen.

Diese Dissertation verfolgte zwei Ziele, welche in 6 Forschungsartikeln behandelt werden. Das erste Ziel war es genauere und standardisiertere Einschätzungen vom Schlafverhalten von Babys zu erhalten durch die Verbesserung der Aktimetrie Analysemethoden. Das zweite Ziel war, Veränderungen im die Entwicklungsverlauf vom Schlaf zu charakterisieren, mit einem besonderen Fokus auf dem Zusammenhang zwischen Schlaf und Darmbakterien.

Das erste Ziel wurde mit drei Forschungsartikeln verfolgt. In Artikel 1 ist ein Review-Artikel, in welchem wir die publizierte Literatur zu Aktigraphieforschung für Baby- und Kinderschlaf untersucht haben. Wir haben die methodische Berichtserstattung verglichen, um hervorzuheben, welche Informationen unterrepräsentiert sind. Ausserdem haben wir Empfehlungen für zukünftige Aktigraphieforschung abgegeben. In Artikel 2 haben wir die Schätzungen von zwei oft verwendeten Algorithmen (Sadeh und Oakley/Respironics) verglichen, welche das Schlaf-Wach Verhalten von Bewegungsdaten ableiten. Wir haben herausgefunden, dass die Resultate sich erheblich unterscheiden. Allerdings haben wir eine standardisierte Pipeline vorgeschlagen, welche die Unterschiede zwischen den zwei Algorithmen deutlich verkleinert. In Artikel 3 haben wir eine Hauptkomponentenanalyse angewendet, um grundlegende Komponenten des Schlafes zu erkennen.

Wir haben 5 Komponenten ausfindig gemacht, welche die Schlafmaturierung im ersten Jahr akkurat reflektieren: *Sleep Day* (misst Tagesschlafdauer und -Regularität), *Sleep Night* (misst Nachtschlafdauer), *Sleep Timing* (misst die Bett- und Schlafzeiten), *Sleep Activity* (misst Wachphasen und Bewegungen während der Nacht) und *Sleep Variability* (misst Variabilität der Nachtschlafdauer und -zeiten über die Messtage).

Für das zweite Ziel schauten wir uns die Schlafentwicklung zwischen Säuglings- und Jugendalter an. In Artikel 3 und 4 haben wir eine grosse Kohorte von 162 Babys mit 3, 6 und 12 Monaten gemessen, mit einer Folgemessung mit 24 Monaten. In Artikel 3 haben wir den Schlaf im ersten Lebensjahr charakterisiert. Wir konnten die zuvor berichtete grosse Variabilität zwischen den Babys bestätigen und fanden gleichzeitig eine sehr grosse Variabilität im gleichen Baby zu verschiedenen Messzeitpunkten. Wir entdeckten, dass es in der *Sleep Activity* bereits bei Babys klare Geschlechtsunterschiede gibt. Zudem haben wir festgestellt, dass *Sleep Day* mit der behavioralen Entwicklung zusammenhängt. In Artikel 4 haben wir zusätzlich die Darmbakterien untersucht. Wir konnten zeigen, dass *Sleep Day* mit der Alpha-Diversität zusammenhängt. Ausserdem fanden wir Assoziationen zwischen *Sleep Activity* und Enterotyp sowie bakterieller Maturierung. Zusätzlich beobachteten wir interessante Verbindungen zwischen sowohl Schlaf als auch Darmbakterien und der Entwicklung. Besonders bemerkenswert war, dass wir einen Zusammenhang mit *Sleep Day* bei 12 Monaten und grobmotorischer Entwicklung mit 24 Monaten fanden. Dies bestätigt unsere Hypothese, dass *Sleep Day* ein Marker der Reifung ist. Ausserdem fanden wir einen positiven Zusammenhang zwischen Alpha-Diversität und früher Entwicklung. Insgesamt konnten wir komplexe und dynamische Assoziationen zwischen Schlaf, Darmbakterien und Entwicklung feststellen.

In Artikel 5 und 6 untersuchten wir die spatio-temporalen Eigenschaften der sogenannten »slow waves«. In Artikel 5 haben wir in einem Datensatz von 29 Kindern und Adoleszenten zwischen 2 und 17 Jahren untersucht, wie sich die »slow waves«ausbreiten. Dabei fokussierten wir uns darauf, wie sich das Ausbreitungsmuster über die Nacht und das Alter hinweg verändert. Wir haben herausgefunden, dass ein Aspekt der Ausbreitung sich nur in einer Altersgruppe über die Nacht verändert: die Ausbreitungsdistanz. Diese nimmt nur bei Kindern unter 5 über die Nacht ab. In Artikel 6 haben wir den Zusammenhang zwischen interzellulären langsamen Os-

zillationen und »slow waves«auf dem Kopf besprochen. Zudem haben wir gezeigt, dass sich der Ursprung der »slow waves«im Laufe der Entwicklung verändert. Bei Kindern ist der Ursprung der »slow waves«weniger häufig in frontalen Regionen als bei Jugendlichen. Zusätzlich haben wir die Hypothese aufgestellt, dass die Ausbreitung der »slow waves«nicht nur die Gehirnreifung widerspiegelt, sondern diese potentiell auch vorantreibt.

Diese Dissertation zeigt die bemerkenswerte Entwicklung des Schlafes von der Kindheit bis zur Adoleszenz auf. Zusätzlich konnten wir erste Hinweise für eine Schlaf-Darm-Verbindung im ersten Lebensjahr zeigen. Wir fanden spannende Assoziationen zwischen Schlaf, Darmbakterien und Entwicklung. Zudem präsentiere ich Vorschläge für methodologische Weiterentwicklungen und potenzielle zukünftige Forschungsfragen, die unser Wissen über die menschliche Entwicklung erweitern könnten.

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CONTENTS


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1

INFANT DEVELOPMENT

It is easier to build strong children than to repair broken men.

— Frederick Douglass

Ideas on healthy infant development have changed throughout humanity's history (Fogel, 2010). Empirical research on infants has become widespread in the last 60 years after it has been shown that newborns perceive and respond to sensory perceptions very early on (Frantz et al., 1962). With this new understanding came the insight that the first few months of life are crucial for learning and establishing the infant-parent relationship (Nugent et al., 2008). Realizing this importance of infancy and childhood for later life, research into infant and child development has started to play a significant role in infant care behavior. While parenting advice and books had already existed for centuries, the advice was now given based on empirical studies rather than theoretical considerations.

However, what is healthy development? What can parents do to give their children the best start in their lives?

Researchers try to approximate this question by linking early life behavior with childhood, adolescent, or adulthood outcomes. However, the selection of predictor and outcome variables is not trivial. Firstly, there are many different development areas, such as cognitive, motor, social, and emotional development (Squires et al., 1995). Moreover, we have to define which outcomes are considered normal for each area and which are not. However, what falls within or outside of the norm is very hard to define. Mostly there are no definite cut-offs, but there is a continuum between healthy and unhealthy behavior. It is also important to note that which outcome is deemed successful is shaped by societal norms. Additionally, there are many behavioral and physiological aspects of infant life. There are many distinct but interconnected domains, which are influenced by environmental factors. It is often the culmination of multiple factors that

result in a risk for later outcomes (Appleyard et al., 2005).

Another issue is the time frame; should we only focus on long-term outcomes to adolescence or adulthood? Is it relevant if some behavior in infancy only influences early childhood but has no consequences in later life?

Ultimately, each study can only address a subset of factors. Like a puzzle consisting of thousands of pieces, each piece is fundamental to understanding early life and its consequences for later outcomes. Moreover, with every piece, the picture becomes clearer. This thesis is one of these puzzle pieces. I will address two critical aspects of early life: first, sleep, the state that is the most common in the first year of life, and secondly, gut bacteria, an array of mostly symbiotic bacteria that make up half the cells in our body. While there is already a large body of literature on sleep in infancy and a growing literature on the effects of sleep in infancy on later outcomes, studying the gut bacteria and their development is a more novel field (Pariente, 2019). We are beginnging to understand how gut bacteria develop, and in the last decade, we have started to appreciate the importance of gut bacteria for healthy development. However, this thesis is the first to examine the association of sleep and the gut bacteria in infancy and their combined effect on developmental outcomes one year later.

While the research in this thesis focuses on understanding associations in healthy development, this work is a vital cornerstone for translational research. In combination with the giants upon which shoulders it stands and the research that will build upon the knowledge generated here; hopefully, this thesis will contribute to our ability to give infants a positive start in their lives.

2

SLEEP

Let's begin by taking a smallish nap or two.
— Winnie the Pooh

Humans spend up to one-third of their whole life asleep. However, never do we sleep more than in infancy, where sleep is the most common behavioral state (Iglowstein et al., 2003). But what is sleep? Sleep can be defined on two levels, either via behavior or via electrophysiology. A species is sleeping on a behavioral level when they have 1) a specific sleeping site, 2) a typical body posture, 3) immobilization and 4) a higher arousal threshold. Additionally, this sleep state has to be 1) rapidly reversible and 2) homeostatically regulated (Flanigan, 1972; Piéron, 1912; Tobler, 1984). On an electrophysiological level, sleep is defined by altered brain electrical activity, which correlates with the behavioral state (Nitz et al., 2002). Sleep behavior is apparent in many different species (S. S. Campbell & Tobler, 1984), but sleep electrophysiology has mainly been examined in mammals and birds. Nonetheless, it has been proposed that sleep is universal, on the basis that we lack clear evidence of animals that do not sleep (Cirelli & Tononi, 2008).

So why do we sleep? Considering the inactivity in sleep, coupled with the inability to fulfill other needs and the heightened state of vulnerability, many researchers have suggested that there must be a universal and core function to sleep (Cirelli & Tononi, 2008; Rechtschaffen, 1998). However, other researchers have challenged this notion by suggesting that sleep's function across all species is ultimately the trivial function of rest (Rial et al., 2007; Webb, 1974). They have not excluded the possibility that there might be secondary functions of sleep in mammals or humans. In mammals, sleep has been linked with restoration of the body (notably the immune system, Imeri and Opp, 2009) and brain (Benington & Craig Heller, 1995) including synaptic homeostasis (Tononi & Cirelli, 2006) and neural waste removal (Xie, 2013). Additionally, in humans, sleep has been proposed to be the ideal state for memory consolidation (Kirov et al., 2009; Rasch & Born, 2013). These are all critical functions for healthy development, though a

specific focus lies on sleep's role within learning and memory (Friedrich et al., 2020; Peiffer et al., 2020). Even more, sleep has specifically been linked to brain maturation and neural reorganization (Cao et al., 2020; Feinberg & Campbell, 2010; Kurth et al., 2010).

When considering sleep in development, it is essential to focus not only on the effects that sleep has on the individual but also consider the whole family unit. The sleep of an infant (or lack thereof) can be a source of depression and stress to parents (Lam et al., 2003; Martin et al., 2007). Additionally, infant sleep practices show cultural variation (Mindell et al., 2010; Sadeh et al., 2011). Therefore, it is imperative to take a multimodal view of sleep that includes cultural and family settings. The goal should be to maximize the well-being of the whole family. While the infants' needs are essential to fulfill, the parents' individual needs also have to be taken into account.

2.1 MEASURING SLEEP

Researchers use both subjective and objective methods to measure sleep. In infants, subjective methods include questionnaires (e.g. the Brief Infant Sleep Questionnaire, Sadeh, 2004) and diaries (Parmelee, 1961) filled out by their carers. Objective methods include actigraphy (Sadeh et al., 1991), videosomnography (Anders & Keener, 1985) and electroencephalography (EEG; Hagne, 1972). All methods have advantages and disadvantages (see Table 1, adapted and expanded from Sadeh, 2015).

There is a moderate agreement between the different methods. There are higher discrepancies between questionnaires and objective data than diaries and objective data. Furthermore, the agreement between diaries and actigraphy is higher than of either with videosomnography (Camerota et al., 2018; Werner et al., 2008). Objective methods should be validated using concurrent EEG measures. This validation should be for the specific population and device. In infants, only some actigraphs have been validated against EEG. Usually, a sufficient or high sensitivity is reported (ability to detect sleep, 67 - 99%) but specificity can be quite low depending on the device and algorithm (ability to detect wake, 39 - 98%, Sadeh et al., 1995; So et al., 2007; Tilmanne et al., 2009). This validation is lacking for videosomnography.

Method	Advantages	Disadvantages
Objective Methods		
Electroencephalography or polysomnography	- Gold standard for sleep research - Detailed information including sleep stages - Clinical diagnosis possible - High standardization of application and scoring - Very high temporal resolution - Complex analyses are possible - Information on brain maturation - Ability to measure spatial differences	- Expensive (equipment and laboratory) - Mostly done in the laboratory (unnatural environment), though more and more portable systems exist - Usually limited to one or two recordings - Often only either nap or nighttime sleep - Scoring is time-consuming, but efforts are made to automate scoring - Can be difficult to perform with infants and young children - More difficult to recruit participants
Videosomnography	- Documents parasomnias - Documents caregiver interactions - Non-intrusive home monitoring - Enables some clinical diagnosis - High resolution	- Requires installation in home - Time-consuming scoring - Privacy concerns - Limited to certain sleep positions and locations - Indirect measure of sleep - No sleep stage information
Actigraphy	- Cost-effective - Long-term recording of sleep/wake behavior - 24 h recording for both day and nighttime sleep - No installation - Scoring is (semi-)automatic - High resolution	- Indirect measure of sleep (via activity) - No sleep stage information - No data on specific behaviours - Artifacts due to external movements, device removal etc., which have to be addressed during scoring - Devices break or malfunction - Might be difficult if child is wcared for externally - Limited standardization of device, algorithms and sleep variables
Subjective Methods		
Questionnaires	- Easy for participants, large sample sizes - Cost effective - Provides unique information on subjective factors - Can address many different situations	- Bias through subjectivity or compliance - Limited to aspects parent's are aware of - Lack of normative values - Low resolution
Diaries	- Cost effective - Long-term recording of sleep/wake behaviour - 24 h recording for both day and nighttime sleep	- Bias through subjectivity - Limited to information parents' are aware of - Time-consuming for parents - Difficulties with external caregivers - Low resolution

Table 1: Advantages and disadvantages of different methods to measure sleep in infants. Adapated after Sadeh, 2015

The choice of method should depend on the research question and resources available while considering all the necessary advantages and disadvantages. However, for many research areas, actigraphy with concurrent sleep diaries has emerged as the preferred method (Meltzer et al., 2012). This combination of objective and subjective data works in large populations and natural environments over prolonged periods while still providing high resolution.

2.1.1 *Actigraphy*

Actigraphy is obtained by measuring acceleration using a movement sensor (actigraph) attached to (most commonly) the wrist or ankle. From the movement patterns, we deduct sleep-wake patterns (Figure 1). For this, an algorithm (e.g., Galland, Kennedy, et al., 2012; Oakley, 1997; Sadeh et al., 1995) estimates sleep or wake dependent on the current episode's movements and accounting for the broader context by including the surrounding episodes. Usually, data is recorded across several days (e.g., Acebo et al., 1999 recommend at least 5 - 7 days), and then the resulting sleep variables are averaged across the recording days.

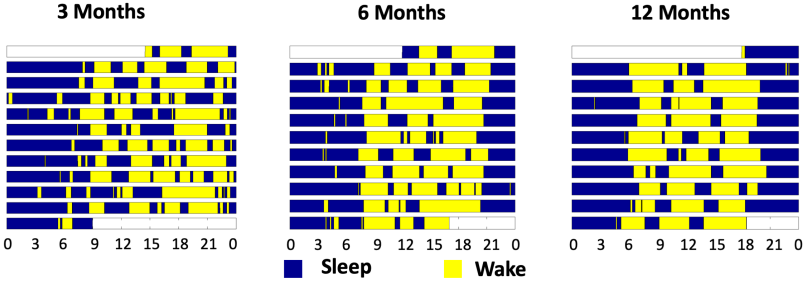

FIGURE 1: Sleep-wake patterns of a single participant at 3, 6, and 12 months of age as derived from actigraphy. Sleep is pictured in blue, wake is pictured in yellow (unpublished data).

Actigraphy is especially prevalent in sleep research of infants and young children because the device is unobtrusive and much more infant and parent-friendly than, for example, EEG. Additionally, sleep in infants is very variable across days; therefore, using just one night as is the standard

with EEG does not result in a reasonable estimation of habitual sleep patterns (James-Roberts & Plewis, 1996). Actigraphy can also be used in clinical practice to detect sleep disturbances in infants (Sadeh, 2015).

One primary problem in the actigraphy research field is the lack of standardization. There are many different devices, different algorithms, and different sleep variables. Often generalizability between studies is further impaired by researchers reporting insufficient details about their methodology. My thesis is addressing some of these issues.

2.1.2 *EEG*

EEG is the measurement of electrical activity in the brain by measuring changes in electric field potentials from pyramidal neurons in the cortex (Buzsáki et al., 2012). We measure these potentials by placing electrodes on the scalp and measuring differences in electrical potential between two electrodes (for sleep data, often a mastoid [electrodes behind the ears] reference is used) or between one electrode and all other electrodes (average reference). The ideal reference would capture all noise but none of the actual brain signals. There are different setups with which researchers arrange the electrodes; the most common is the 10-20 system (Jasper, 1958). However, using more electrodes - so-called high-density EEG (hdEEG) - is beneficial because it gives a higher spatial resolution of the scalp signal. High-density systems usually employ 64, 128, or 256 electrodes in an equidistant distribution across the scalp. Figure 2 shows the layout for a 128 electrode net. The same layout is used for infants, except that the electrodes in the face are removed (electrodes 125-128).

EEG generally has a high temporal resolution but a low spatial resolution. While hdEEG provides a higher resolution of measurement on the scalp, it does not solve the inverse problem, which means that we rely on source localization to estimate the signal's origins (Srinivasan et al., 2006).

Polysomnography supplements the EEG with the electrooculogram (EOG), which measures eye movements, and the electromyogram (EMG), which measures muscle activity. Other physiological measures can be added, depending on the research question. With hdEEG, EOG and EMG can be captured by the electrodes on the net.

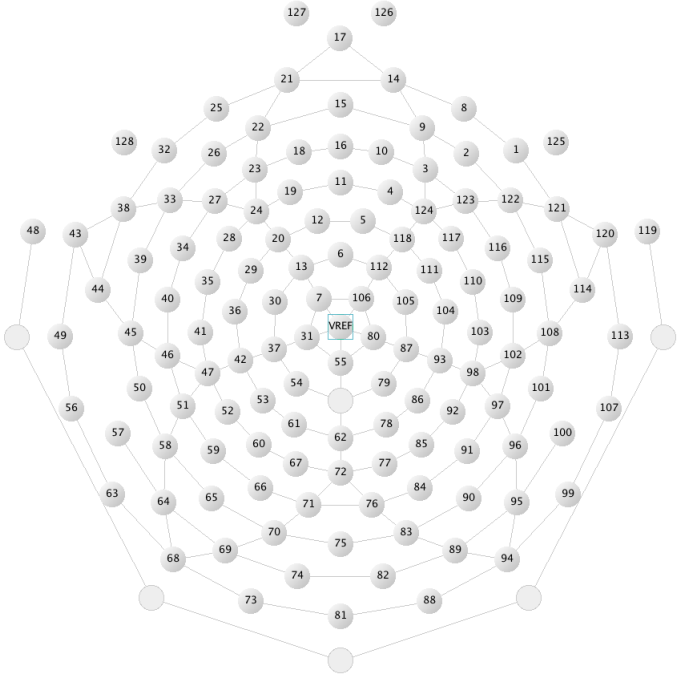

FIGURE 2: The 128 electrode layout (top view) used by the Geodesic Sensor Net (Electrical Geodesics, Inc., EGI) taken from the Geodesic Sensor Net Technical Manual.

Experts score sleep per 20 s or 30 s epochs following rules established in scoring manuals. Most commonly, the American Academy of Sleep Medicine rules are used (Iber et al., 2007). Sleep is divided into rapid eye movement sleep (REM sleep) and non-rapid eye movement (NREM) sleep. Furthermore, NREM sleep is divided into three stages; N1 (light sleep), N2 (stable sleep), and N3 (deep or slow-wave sleep). In infants, these sleep stages can be scored and recognized after around 4-5 months of age. Before, researchers do not distinguish the different NREM stages but only score NREM/REM sleep, which are also called quiet and active sleep (Marquis, 1933).

The distinction of the sleep stages is based upon the rhythms in the EEG. Specific EEG oscillations such as slow waves (waves of frequency 0.5 - 4 Hz and peak-to-peak amplitude > 75 μv) or spindles (a train of distinct waves

with an 11-16 Hz frequency and duration of more than 0.5 s) are specific to certain sleep stages (Iber et al., 2007). Figure 3 shows an overview of the typical EEG patterns of each stage.

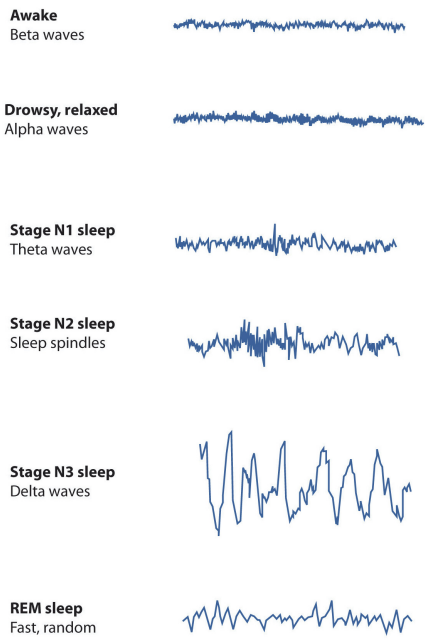

FIGURE 3: The typical EEG pattern for each sleep stage from "Sleeping and Dreaming Revitalize Us for Action", section 5.1 from the book Beginning Psychology (v. 1.0) (Stangor, 2012)

We can characterize sleep EEG in many different ways. The following analyses are often reported (non-exhaustive list) 1) sleep stages (e.g., the percentage spent in different sleep stages), 2) EEG power within specific frequency ranges (e.g., slow wave activity), 3) Specific EEG oscillations (e.g., spindle density), 4) Spatio-temporal distributions across the scalp (e.g., the traveling sleep slow wave).

2.2 SLEEP IN DEVELOPMENT

Sleep changes vastly across the whole lifespan (see Figure 4). Not only does sleep duration decrease across the lifespan, but also the composition of NREM and REM sleep changes. I will focus on changes in infancy and childhood through adolescence in the next two sections.

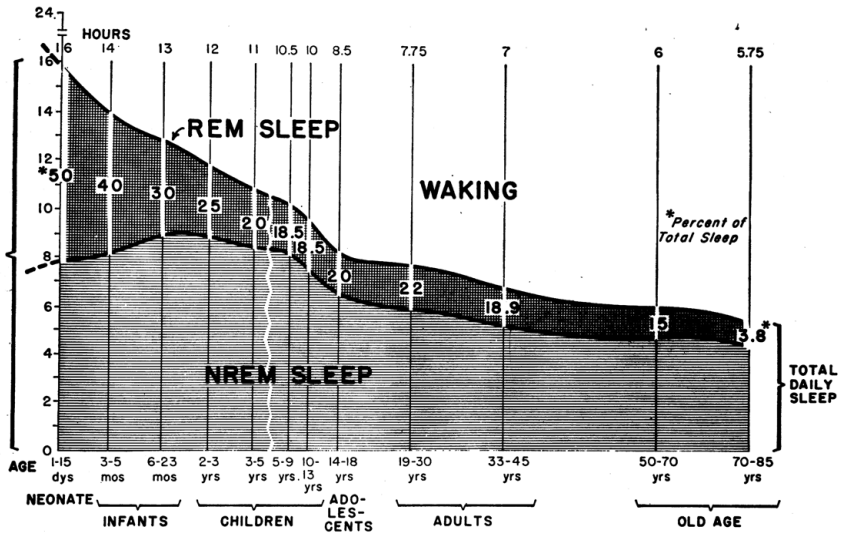

FIGURE 4: The change of sleep duration and sleep stages across the life span, from Roffwarg et al., 1966. Reprinted with permission from AAAS.

2.2.1 *Changes in the first year of life*

In infancy, sleep changes drastically over a short period. The change from an equal distribution of sleep phases across the day (polyphasic sleep) to a consolidated night-time sleep phase (monophasic sleep) starts in the first year of life and continues to around 3-5 years of age (Iglowstein et al., 2003). By 1-year of age, sleep is consolidated to one long night-time sleep phase with one to two naps during the day (Figure 5). The 24-h rhythm emerges at around four weeks of age (Shimada et al., 1999). Once the rhythm is established, many other aspects of sleep start to change, e.g., the

consolidation of night-time sleep (fewer periods awake). Additionally, sleep quantity, measured as total sleep duration across 24 h, decreases by about 8 minutes per month in the first year of life (Galland, Taylor, et al., 2012).

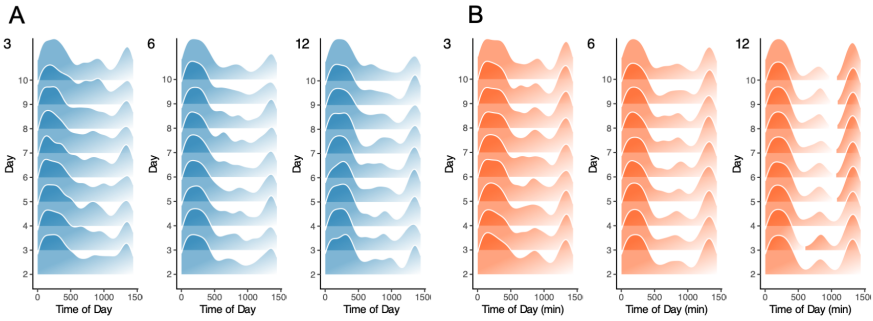

FIGURE 5: The density of sleep (likelihood of being asleep) at 3, 6, and 12 months of age across complete assessment days from the ten infants with the highest (blue) and the ten infants with the lowest day-to-day variability (red) at 3 months of age. In both groups, sleep gets more regular and consolidated (more similar across days and defined peaks). However, at 12 months, there are still differences between the two groups (more defined peaks in the red group, Unpublished data).

Establishing normative sleep values for infants is much harder than for children or adults due to the large variability between infants (Iglowstein et al., 2003). Especially at the beginning of life, this variability is enormous: some infants sleep 19 h per day in the first few months while others only sleep 9 h per day. Therefore, it is hard to establish a cut-off of what is still within the normative range and when the sleep pattern should be concerning. Additionally, the multidimensional nature of sleep makes it necessary to have normative values for total sleep duration and separately for day- and night-time sleep and other aspects such as sleep timing and consolidation.

Not only the behavioral aspects of sleep undergo a drastic change, but there is also a change in the composition of sleep. Just after birth, REM makes up nearly half of the total sleep time, decreasing to 30% until 12 months of age (Figure 4, Jenni et al., 2004; Sankupellay et al., 2011). Infants

have a shorter NREM/REM cycle duration of approximately 45 to 60 minutes, compared to the 90 minutes seen in adults (Hoppenbrouwers et al., 1988; Jenni et al., 2004). The EEG spectrum also shows clear maturational changes in the first year of life: in the first six months, there is an increased spectral power in frequencies below 10 Hz and above 17 Hz (Jenni et al., 2004), which might reflect the increase in grey matter (Knickmeyer et al., 2008). Additionally, the characteristics of adult NREM sleep start to emerge, sleep spindles start to appear from 2-3 months of age (Jenni et al., 2004), K-complexes (a well-delineated negative sharp wave immediately followed by a positive component of more than 0.5 s of duration) appear between 4-6 months old (Metcalf et al., 1971) and slow waves are present from 2 - 4 months on (Fattinger et al., 2014; Jenni et al., 2004).

The topographical distribution of the EEG power within the delta frequency (1 - 4 Hz) shows an unspecific activation pattern at birth with a shift towards occipital maxima at three months of age. Additionally, the EEG power within higher frequencies all show a central maximum at three months of age (Guyer et al., 2019). At six months of age, we found the same occipital maxima for the delta range. However, for the theta range (4.75 - 7.75 Hz), we saw maxima in both the central and occipital areas, interestingly more similar to what Guyer et al. saw in the preterm infants at three months of age. Figure 6 shows the mean distribution of EEG power within the delta, theta, alpha, and sigma frequency range at six months of age.

2.2.2 *Changes in Childhood and adolescence*

Sleep maturation continues throughout childhood and adolescence. The habit of daytime napping continues into early childhood: most children stop napping between 3 (50% napping) and 5 years of age (8 % napping) (Iglowstein et al., 2003). Once napping ceases, another drastic shift starts to happen: social jetlag, the misalignment of weekday and weekend bedtimes, starts to emerge. A recent cross-sectional study with infants, children, and adolescents from 0 - 25 years old identified two breakpoints: at 5 - 7 years old, the social jetlag starts and 15 - 17 years old, the social jetlag stabilizes and the increase of eveningness stops (Randler et al., 2019). This social jetlag is specifically strong in adolescence because, despite the shorter sleep times, sleep need itself does not decline across adolescence (Ohayon et al.,

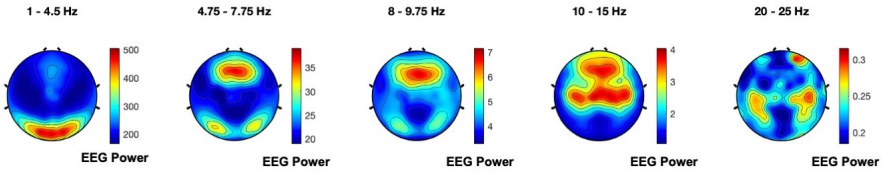

FIGURE 6: Topographic map of the absolute power density in the first 30 mins of NREM sleep across the delta (1 - 4.5 Hz), theta (4.75 - 7.75 Hz), alpha (8 - 9.75 Hz) and sigma (10 - 15 Hz) frequency bands in 6 months old infants (n = 31). Absolute power density is color coded (maxima in yellow, minima in blue) and bars indicate values in $\mu V^2/s$. We applied the following standard preprocessing: bandpass filter 0.5-50 Hz, down-sampling to 128 Hz, average referencing, sleep stage scoring, semi-automated artifact rejection, spectral analysis with Fast Fourier transform routine (unpublished data).

2004). The social jetlag is also due to the change in chronotype (a person's preference to sleep at a specific time of the day): most children have an early chronotype (Randler & Truc, 2013) but this shifts to a late chronotype beginning at around 12 - 14 years of age (Randler et al., 2009).

Scholle et al., 2011 report the cross-sectional sleep data of 209 children from 1 - 16 years of age. Based on their data, we can establish the following: Total sleep time at night shows an inverted u-shape; it increases from 1 - 3 years old and then decreases. Wake after sleep onset reduces from 1 to 3 years and then stays consistent. N2 increases steadily from 2 years old, while N3 and REM decrease across the whole age spectrum. The length of the NREM-REM sleep cycles continues to increase until they reach 90 minutes at around 16 years of age.

A recent study suggests that the function of sleep changes across development (Cao et al., 2020). Based on computational models and a meta-analysis of data across development, the authors suggest that the function of sleep changes between 2 - 3 years of age from a primary focus on brain maturation and restructuring to repairing and removal of neural waste products.

The sleep EEG in childhood and adolescence also shows interesting maturational patterns. Sleep EEG maturation seems to mirror brain maturation processes. EEG coherence (the similarity between two electrodes) increases in childhood (Kurth et al., 2013) and adolescence (Tarokh et al., 2010), similar to white matter development (Huttenlocher & Dabholkar, 1997). Slow wave amplitude shows an inverse u-shape peaking around ten years of age (Feinberg, 1982), a very similar progression to synaptic density (Huttenlocher & Dabholkar, 1997) but also grey matter development (Giedd et al., 1999). Spindles undergo an interesting developmental pattern; after emergence in the first year, they decrease in prevalence until two years old, remain absent until around 4.5 years, and then increase in prevalence again (Tanguay et al., 1975) and keep increasing throughout adolescence (Hahn et al., 2020). Furthermore, the topographical distribution of spectral power of slow wave activity mirrors cortical development (Kurth et al., 2010), with young children showing an occipital maximum, while from 14 - 17 years old and across adulthood, the maximum lies frontally. The maturation of synaptic density shows the same pattern; it also matures in occipital regions first and last in frontal regions. Slow wave activity in sleep reflects the depth of sleep but is also use-dependent (i.e., increases most in brain areas used during wake; for a review, see Siclari and Tononi, 2017). Interestingly, the maturation of slow wave activity preceded the maturation of skills across childhood and adolescence by around 3.5 years, and the maturation of slow wave activity predicted skills (Kurth et al., 2012). This suggests that sleep a) may be vital for normal brain development, and b) can function as a biomarker for developmental diseases. Sleep slow waves travel across the cortex, spreading out from their origin (Massimini, 2004). Interestingly, the traveling behavior changes across development, with slow waves traveling further with age (Kurth et al., 2017). The traveling behavior is also associated with white matter myelin.

Therefore, infancy, childhood, and adolescence are associated with significant sleep changes both on a behavioral and a neurophysiological level. These changes likely reflect brain maturation. Additionally, some evidence is emerging that they might even influence brain maturation.

2.2.3 *Effects of early sleep on later outcomes*

There has been significant interest in understanding how early sleep behavior predicts later behavioral and psychosocial development and health outcomes. This prediction could potentially hint towards a causal role for sleep in development, but sleep could also be a concurrent symptom for developmental disorders and therefore serve as a biomarker. However, the results on whether sleep predicts later outcomes have been very mixed, especially for sleep behavior in the first year of life. The following section will reflect on results from previous studies with examples from a) behavioral and neurodevelopmental outcomes, b) mental health outcomes, and c) health outcomes.

2.2.3.1 *Infancy*

Behavioral outcomes

Persistent early sleep problems (as reported by parents) have been shown to predict slightly higher behavior problem scores in childhood (Lam et al., 2003). However, when using a longer follow-up duration, these effects disappeared (Price et al., 2012b), and similarly, no long-term effects of a sleep intervention were found (Price et al., 2012a). Another study found no association between sleep behavior in infants 3 - 13 months of age and behavioral development (Mindell & Lee, 2015). It is important to note that all these studies used parental reports rather than objective sleep assessments. One study using objective sleep data found that early sleep duration was linked to behaviors symptomatic for autism spectrum disorder at 18 - 24 months of age, but only for girls (Saenz et al., 2015). A review of the effects of early sleep on cognitive, psychomotor, and temperament development concluded that the literature generally suggests links between sleep and daytime functioning. However, they also pointed out methodological problems, including inconsistencies in sleep parameters, lack of control variables, and different assessment timing between studies (Ednick et al., 2009).

Health outcomes

An early intervention for infant sleep problems has shown no influence on weight at six years old (Wake et al., 2011). However, another study highlights the importance of the exact definition used to define sleep problems in infants, affecting the results (Alamian et al., 2016). They found associations

between infant sleep problems at 6 and 15 months and overweight at 11 years old, but only when using 2 of 3 potential definitions.

2.2.3.2 *Childhood and Adolescence*

Behavioral and neurodevelopmental outcomes

In 3-year-old children, persistent sleep problems were linked with worse outcomes at six years old for aggression, social and attention problems (Simola et al., 2014). Interestingly, insufficient sleep might explain the link between screen time and behavioral problems in 3-6 years old (Kahn et al., 2020). In childhood, sleep problems have been linked to attention-deficit/hyperactive disorder (ADHD), with the severity of ADHD being a risk factor for both transient and persistent sleep problems (Lycett et al., 2014). However, the link between ADHD and sleep disorders might partially be explained by comorbidities and medication (Corkum et al., 1999). It is, therefore, hard to disentangle which effects are directly related to the disorder. Additionally, most of these studies rely on parental reports. When using actigraphy as an objective measure of sleep, most sleep differences between children with ADHD and healthy controls could not be verified (Corkum et al., 2001). This finding suggests that there might be a bias in parents potentially due to behavioral difficulties at bedtimes. Sleep problems in childhood have also been linked with the autism spectrum disorder; however, in a large population study, it was found that sleep disorders do not precede or worsen autistic symptoms, but rather sleep problems were concurrent and worsened due to autism (Verhoeff et al., 2018).

Mental health outcomes

The study from Simola et al. also found higher anxious and depressed mood at 6 years old in children with persistent sleep problems at 3 years (Simola et al., 2014). Sleep problems in 5-9-year-old children have been associated with neuropsychological functioning during adolescence and adult anxiety disorders (Gregory et al., 2005; Gregory et al., 2009). Another study found that early sleep (specifically low rhythmicity and motor activity during sleep) was associated with childhood and adolescent-onset, but not adult-onset anxiety and dysthymic disorders (Ong et al., 2006). Interestingly, a study found that sleep problems in older children and adolescents are predicted by and predictors of mental health issues (Shanahan et al., 2014), suggesting a bidirectional relationship. However, a study in twins

examining sleep problems and depression at ages 8 and 10 found that only sleep problems were predictive of later depression and not vice versa (Gregory et al., 2009). Potentially the bidirectionality of the relationship is age-dependent.

Health outcomes

Children with persistent sleep problems at 3 years also had more somatic complaints and medical risks at 6 years (Simola et al., 2014). Another significant interest has been the link between short sleep duration and later obesity. However, the results have been inconclusive. A study investigating the sleep between 3 - 5 years of age found that longer sleep duration was linked with lower BMI (Carter et al., 2011). Another study examining sleep at six months - 2 years found that sleep durations shorter than 12 hours were a risk factor for being overweight at three years (Taveras et al., 2008). A large population-based study in children and adolescents between 6 - 17 found an association between insufficient sleep and BMI; however, control variables such as race, gender, family income, and household education explained the association (Hassan et al., 2011). Potentially, reported associations in other published studies could also be explained by third variables, which influence sleep and obesity. However, a recent meta-analysis found support for an effect of sleep duration on obesity risk from infancy to adolescence (Miller et al., 2018), though again, most included studies relied on self or parental reports.

Overall we can conclude that there is likely an association between sleep in childhood and adolescence and later behavioral and health outcomes, especially anxiety and mood disorders and obesity. However, especially with objective data, there have been some inconsistent results (Sadeh et al., 2014). This inconsistency highlights the need for more studies that use a) longitudinal design, b) large sample sizes, and c) objective measures. This thesis addresses this gap by measuring sleep longitudinally in a large infant cohort and using both actigraphy with diaries and EEG.

3

GUT BACTERIA

What you see is that the most outstanding feature of life's history is a constant domination by bacteria.
— Stephen Jay Gould

The human body is not only made up of our human cells but also harbors many other microorganisms such as viruses and bacteria. The newest estimates lie at a 1:1 ratio between human cells and microorganisms in the body (Sender et al., 2016). On a genetic level, we are predicted to be outnumbered by a factor of 50 - 100. As we have evolved as hosts of microorganisms, these organisms provide us with metabolic functions that we never evolved ourselves (Bäckhed et al., 2005). The organ with the highest density of microorganisms is the large intestine (together with the lower intestine, also collectively referred to as the gut). The three most common phyla of bacteria found in the adult gut are *Firmicutes* (79%), *Bacteroidetes* (17 %) and *Actinobacteria* (3 %) (Tap et al., 2009).

3.1 MEASURING GUT BACTERIA

3.1.1 *The history of measuring gut bacteria*

While the existence of human bacteria has been known since Antonie van Leeuwenhoek described five different bacteria in his mouth in 1683, it took nearly two hundred years until the germ theory of disease was proposed by Louis Pasteur (Pariente, 2019) and extended by Robert Koch (Loeffler, 1884). Because most gut bacteria cannot grow in oxygenated environments, the research could only take off in the 1940s and 1950s when methods to culture for anaerobic bacteria were discovered (Hungate, 1950). However, many gut bacteria do not thrive in cultures; therefore, research was still limited. In the 1960s, the research with germ-free mice (mice born and growing up in a sterile environment) and the transplantation of bacterial cultures to germ-free mice highlighted the importance of gut bacteria for normal development (Schaedler et al., 1965). In 1996 Wilson and Blitchington used

16S rRNA sequencing on a human fecal sample, which was the beginning of a renewed interest in gut bacteria (Wilson & Blitchington, 1996). This interest was amplified by the invention of next-generation sequencing, which replaced labor-intensive and expensive Sanger sequencing and made gut bacteria analysis accessible for many more researchers. In 2010 another important step was made when a human gut microbial gene catalog was published (Qin et al., 2010). These catalogs allow the association of rRNA fragments to specific bacteria species or genera.

3.1.2 *16S rRNA sequencing and shotgun sequencing*

Most current studies on the gut bacteria analyze stool samples (and are therefore indirect). Researchers extract the genetic content using 16S rRNA sequencing or shotgun sequencing, which allows for taxonomic and phylogenetic analysis of complex microbial communities. 16S rRNA can sometimes not resolve closely related species, while shotgun sequencing gives an exact picture of present and absent genes. However, shotgun sequencing is more expensive and computationally intensive. Nevertheless, if the research should go beyond describing which bacteria are present and into functional metabolic activities, shotgun sequencing is needed. Several different omics approaches have been developed based on shotgun sequencing, such as proteomics (Klaassens et al., 2007) and metabolomics (Jansen et al., 2004), which look at the genes responsible for proteins or metabolomes.

As 16S rRNA is used in this thesis, I want to highlight some advantages and disadvantages of this method.
Advantages:

- Cost-effective
- Computationally efficient
- Allows research of unculturable species
- Limited problem of contamination of Host DNA
- Sensitive for low input

Disadvantages:

- Indirect measurement of gut bacteria

- Absolute quantities are unknown
- Not reliable on species/strain level
- Chimera generation (mixed DNA) and sequencing errors
- Reliance on completeness of referential databases
- No functional profiles
- Limited to bacteria

3.1.3 *Quantification of the gut bacteria*

There are different measures to quantify gut bacteria. Often, researchers are interested in measuring diversity. Diversity can relate to different scales, 1) alpha diversity reflects the diversity within each habitat (e.g., each within each person), 2) beta diversity reflects diversity between habitats (e.g., the similarity between people), and 3) gamma diversity reflects total diversity within a landscape (e.g., within the whole sample). There are several different indices to quantify the different diversities (e.g., the Shannon index for alpha diversity).

Next to diversity measures, scientists have tried to group humans according to their gut bacteria composition into different so-called "enterotypes". In adults, three enterotypes were found, named after the most prominent genus: *Bacteroides*, *Prevotella* and *Ruminococcus* (Arumugam et al., 2011). A study with infants suggested that enterotypes are developed between 18 and 36 months (Bergstrom et al., 2014) . At 1 year old Carlson et al., 2018 suggest that *Bacteroides* and *Ruminococcus* enterotypes are already present, but that the third enterotype is likely based on the abundance of *Faecalibacterium*. A study with infants suggested using enterotypes based on phyla rather than genera and suggested 3 enterotypes in infants which highly depend on geography: *Proteobacteria*, *Actinobacteria* or *Firmicutes* (Kuang et al., 2016). However, there has been some critique about the use of enterotypes because it oversimplifies the diversity of gut bacteria compositions and neglects the continuum between the enterotypes (Jeffery et al., 2012).

Other analyses focus on the abundance and prevalence of specific taxa. An often used marker is the Firmicutes/Bacteroidetes ratio, which has been associated with obesity (Koliada et al., 2017) and type 2 diabetes (Hwang et al., 2015). Yet, other studies have not been able to reproduce these findings

(see Magne et al., 2020 for a review), and it has been found that analysis choices greatly influence these findings (Bahl et al., 2012; Vebø et al., 2016).

Ideally, one should use a combination of measures to reflect the complex gut bacteria data adequately. Researchers and readers alike should be aware of the influence of analysis choices on results and generalizability (Schloss, 2018).

3.2 MATURATIONAL CHANGES IN GUT BACTERIA

The most prominent hypothesis about the colonization of humans by bacteria is the "sterile womb" hypothesis, which postulates that the human womb is sterile and that colonization starts with birth. Recently evidence has emerged that some bacteria are already present in utero ("in utero colonization"). Some authors suggested that these results were due to contamination of probes (for an overview of both hypotheses, see Perez-Muñoz et al., 2017). More research is needed to establish if there is already colonization in utero. Regardless, birth is an important colonization event, and differences in the gut bacteria composition have been reported depending on the birth type (c-section vs. vaginal births, Dominguez-Bello et al., 2010). Infants with a vaginal birth had gut bacteria compositions more similar to their mother than other mothers, while this was not found for babies born via c-section (Bäckhed et al., 2015; Dominguez-Bello et al., 2010). It is still unclear how long the difference between the birth modes persists, with some cross-sectional studies reporting differences still apparent in childhood and even young adulthood (Nagpal & Yamashiro, 2018; Salminen et al., 2004). However, in some longitudinal studies, no differences were detected after six months (Kabeerdoss et al., 2013; Nagpal & Yamashiro, 2018). A recent review suggests that the differences disappear after six months of life (Rutayisire et al., 2016), but more studies will be necessary to explain these differing results from cross-sectional and longitudinal studies.

The transfer from mother to infant occurs at birth via the vaginal bacteria and later in life via other body sites. A recent study has suggested that maternal gut bacteria is the origin of most transmitted strains (Ferretti et al., 2018). During the first year of life, the infant gut bacteria undergo large changes shaped by the changes in the gut environment, and feeding (Bäckhed et al., 2015 see Figure 7). In the first few weeks, the infant gut bacteria show low alpha diversity and high beta diversity. The dominant

families are *Enterobacteriaceae* and other facultative anaerobes (Nagpal & Yamashiro, 2018; Palmer et al., 2007). Over time, alpha diversity increases, beta diversity decreases (Bäckhed et al., 2015). Between 3 and 6 months strict anaerobes dominate the composition including *Bifidobacterium* and *Bacteroides* (Bäckhed et al., 2015; Nagpal & Yamashiro, 2018). After the cessation of breastfeeding, the composition diversifies (Bäckhed et al., 2015; Mancabelli et al., 2020), and between 1 and 3 years old, a bacterial composition similar to adults is reached (Bäckhed et al., 2015; J. E. Koenig et al., 2011). The composition at one-year-old is more similar to adults than young infants but functionally not completely matured (Bäckhed et al., 2015). The functional maturation possibly lasts into childhood (Hollister et al., 2015).

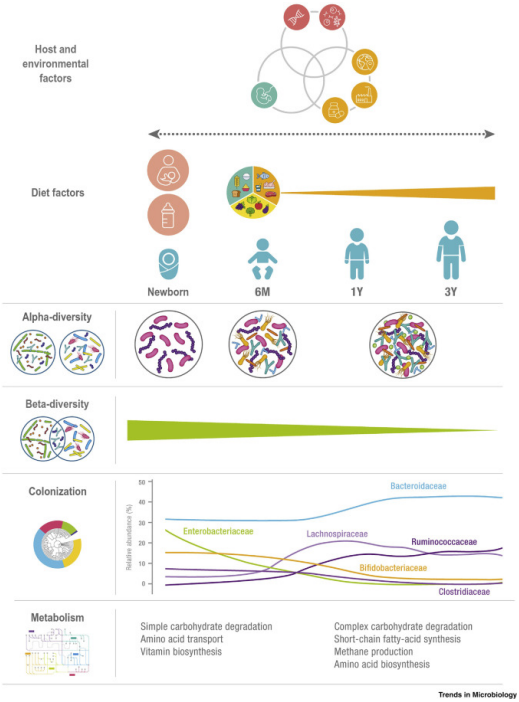

FIGURE 7: The development of the gut bacteria in the first 3 years of life, from Derrien et al., 2019 doi: 10.1016/j.tim.2019.08.001 © Creative Commons Attribution-NonCommercial-No Derivatives License (CC BY NC ND). Alpha diversity increases, beta diversity decreases with differences due to diet and environmental factors including birth mode

3.3 THE GUT-BRAIN AXIS

The English language (and many other languages) acknowledges the association between the gut and emotions in terms like "gut feeling" or "gut-wrenching decisions". Furthermore, the concept of a gut-brain link has a long history, with the first scientific investigation by William Beaumont in the 1800s (Beaumont & Osler, 1833). Only in the last two decades have we come to appreciate the gut bacteria's role in this process (also termed the microbiota-gut-brain axis; for a comprehensive review, see Cryan et al., 2019). Research with germ-free mice increasingly identifies how gut bacteria affect the brain (Clarke et al., 2013; Gareau et al., 2011; Hegstrand & Hine, 1986) and how antibiotics disturb gut-brain interactions (O'Mahony et al., 2014). Beyond that, emerging research lines demonstrate the effects of probiotic intake on stress regulation, mental health, and brain activity in human adults (Allen et al., 2016; Pinto-Sanchez et al., 2017; Tillisch et al., 2013). The early developmental period seems to be especially critical for the gut-brain axis, as both the brain and the gut undergo major maturational changes. The human brain doubles in volume as grey matter increases (Knickmeyer et al., 2008). Additionally, there is an enormous functional restructuring starting in the primary sensorimotor network and ending within the executive control networks (Gao et al., 2015). This dynamic restructuring is concurrent with the large maturational changes within the gut bacteria. The importance of early developmental periods is highlighted by findings that only early, but not late, recolonization of germ-free mice reverses neurodevelopmental disruptions (Heijtz et al., 2011; Sudo et al., 2004). Therefore it has been suggested that there are "sensitive" periods in early childhood where the gut-brain axis prompts normal neuronal development.

3.4 EFFECTS OF EARLY GUT BACTERIA ON LATER OUTCOMES

Due to the likely "sensitive" periods of the gut-brain axis in early childhood, there is an influence of the early gut bacteria on later outcomes. As gut bacteria have been associated with many diseases (e.g., Inflammatory Bowels Disease Nishida et al., 2018, multiple sclerosis Jangi et al., 2016; Shahi et al., 2017, see Figure 8 for an overview), it is likely that some of these associations already start in infancy.

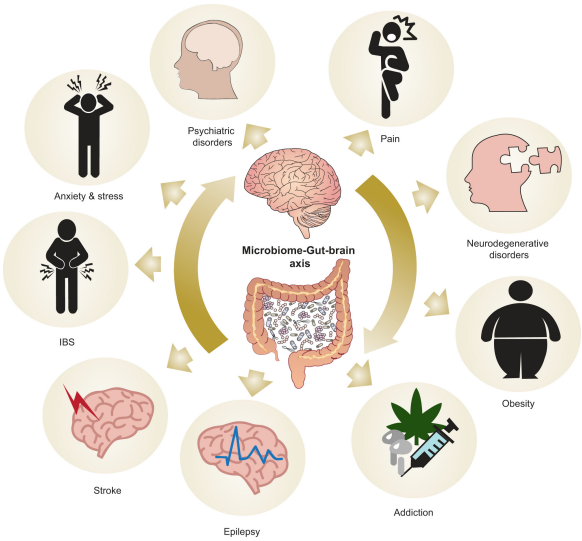

FIGURE 8: Previously reported associations of the gut bacteria and health. From Cryan et al., 2019 © The American Physiological Society, reprinted with permission.

Gut bacteria shape the immune system's development in infancy and are likely to play a role in autoimmune diseases (Cerf-Bensussan & Gaboriau-Routhiau, 2010; Torres et al., 2020). For example, it has been shown that infants developing an allergy until five years of age show altered gut bacteria composition in the first two months of age (Sjögren et al., 2009). Interestingly, another study found that the gut bacteria composition at one week and one month but not at 12 months was related to later asthma, potentially suggesting an early sensitive period. However, this study is based on a small sample (Abrahamsson et al., 2014).

Gut bacteria in adults had been linked to obesity, and the transfer of gut bacteria from an obese human donor to germ-free mice leads to more weight gain than transfer from a lean donor (Ridaura et al., 2013). Studies have shown that infants delivered by c-section and infants with antibiotic exposure have an increased risk of being overweight in later life (Azad et al., 2014; Huh et al., 2012). However, another long prospective study found no association between c-section and later overweight but found that early antibiotic use was associated with later overweight. Interestingly, in

infants from normal-weight mothers, antibiotic use was associated with an increased overweight rate, while it was decreased in infants from overweight mothers (Ajslev et al., 2011). A study in mice has shown that early antibiotic use can lead to perturbations in the gut bacteria that have long-lasting consequences, significantly enhancing the effects of diet-induced obesity (Cox et al., 2014). Nevertheless, gut bacteria likely also show a role in growth impairment due to malnourishment, as gut bacteria from malnourished infants and children lead to impaired growth and metabolic abnormalities. These effects were greater when gut bacteria from younger infants were used (Blanton et al., 2016).

Gut bacteria have also been associated with neurodevelopmental disorders and mental health issues, likely due to the gut-brain axis disturbances. For example, more and more evidence is emerging for the gut bacteria's role in Autism Spectrum Disorder (Strati et al., 2017), with first studies showing positive effects of probiotics on symptoms (Y.-W. Liu et al., 2019; Pärtty et al., 2015). Laue et al. (2020) reported that the gut bacteria composition at 1, 2, and 3 years of age was associated with social behaviors related to autism at age 3. Potentially, there is an association between early gut bacteria and later anxiety due to the gut bacteria's influence on the hypothalamic-pituitary-adrenal axis (O'Mahony et al., 2014); however, thus far, this has only been shown in rats. For cognitive outcomes, a first study showed a link between gut bacteria composition in infancy and later cognitive outcomes and potentially gray matter maturation (Carlson et al., 2018).

More studies using longitudinal designs and objective gut bacteria data from infancy are needed to understand these associations. Additionally, it is essential to keep in mind that much of the research thus far is based on associative differences between healthy and diseased populations. These studies give no insight into causality, and a causal role of the gut bacteria has not been proven in any of these diseases (including Inflammatory Bowel Diseases Ni et al., 2017). Studies that transplant gut bacteria from healthy and unhealthy hosts into germ-free mice provide more explicit evidence for causality. However, since germ-free mice are a) not healthy and b) the typical composition of gut bacteria varies between species (Lyon, 2018), these studies should be interpreted with caution. Additionally, the conceptualization of illnesses in animals that do not naturally show these illnesses can be problematic. Along the same lines, establishing what a healthy gut bacteria composition is, has proven to be challenging because of

the large variability due to age, geography, and other factors (Greenhalgh et al., 2016). More functional analyses of gut bacteria are needed to establish what bacteria are involved in processes that contribute to human health. However, it has become evident that gut bacteria are key players in human health and can function as biomarkers for disease. Potentially, reducing gut bacteria dysbiosis can alleviate some illnesses.

In sum, gut bacteria are tightly linked to health and disease, but more studies are needed to understand the exact etiology. Special consideration should be given to early developmental periods, as these ensure healthy, normal maturation.

4

THE SLEEP-GUT AXIS

They eat, they crap, they sleep, and if they're crying they need to do one of the three and they're having trouble doing it. Real simple.

— Matthew McConaughey

Interestingly, gut bacteria undergo circadian rhythmicity (Paulose et al., 2016), and their rhythms might influence host circadian rhythms (Leone et al., 2015) or vice versa (Liang et al., 2015). Gut bacteria have been linked to sleep in rodents, such that depletion of gut bacteria in rats modifies slow wave sleep (Brown et al., 1990). In humans, gut bacteria have been linked to sleep disturbances, sleep quality, and sleep duration (González-Mercado et al., 2020; Grosicki et al., 2020; Smith et al., 2019). Both sleep timing and sleep duration influence the gut bacteria: deregulated sleep has been shown to alter gut bacteria composition (Anderson et al., 2017; Z. Liu et al., 2020; Poroyko et al., 2016; Thaiss et al., 2014; Voigt et al., 2014; Voigt et al., 2016), and insufficient sleep has metabolic consequences that lead to bacterial alteration and translocation (Benedict et al., 2016; Bowers et al., 2020; Everson & Toth, 2000; Maki et al., 2020) (yet Zhang et al., 2019 reported null results). The link seems bi-directional: alterations of gut bacteria by dietary pre- and probiotics have been shown to modify sleep consolidation in mice and rats (Miyazaki et al., 2014; Thompson et al., 2020; Yu et al., 2020). Similarly, probiotics have been shown to alleviate subjective and objective sleep disturbances related to stress in humans (Takada et al., 2017). A link was proposed between dysbiosis of the gut and the deadly effect of sleep deprivation via an increase in an enzyme (Nox) (Iatsenko et al., 2018), which in turn produces reactive oxygen species that can trigger oxidative stress and potentially death (Vaccaro et al., 2020). It is important to note that most studies in humans use small sample sizes, and findings have to be confirmed in more extensive studies. However, there is likely a link between sleep and gut bacteria, and this link seems to be bi-directional. Thus far, no studies have looked at the sleep-gut axis in infancy or childhood. It is, therefore, unclear when the sleep-gut axis develops. Considering the significant developments of both sleep and gut

bacteria in infancy, I hypothesize a concurrent maturation. I suggest that more mature sleep patterns are linked to more mature gut bacteria profiles and vice versa, likely because they influence each other bi-directionally.


To Do
Sleep
eat
Do science

5

AIMS

The aim of science is not to open the door to infinite wisdom, but to set a limit to infinite error.
— Bertolt Brecht

My thesis illuminates how sleep develops in infancy and childhood. Furthermore, I relate sleep maturation to the maturation of gut bacteria and behavioral development, both concurrently and as an outcome at two years of age. To accomplish these aims, I address specific problems of measuring sleep in infancy with actigraphy. Therefore, this thesis pursues two overarching goals: improving infant sleep research methodology and improving knowledge on sleep in development. This chapter will characterize all my contributions and how they related to my thesis' overarching goals.

1. Improve standardization of infant sleep research using actigraphy

   Actigraphy is popular in infant sleep research and has many benefits, including the possibility to measure sleep objectively in natural settings over long periods. However, one considerable drawback is that actigraphy research lacks standardization compared to EEG sleep research. This lack of standardization affects many parts of actigraphy research, including the use of many different devices, analysis algorithms, sleep variables, placement of devices, and recording settings. Due to this large variability, it is essential that researchers accurately and exhaustively report their methodology.

   a) Characterize adherence to reporting standards

      Article 1: Actigraphy in sleep research with infants and young children: Current practices and future benefits of standardized reporting
      Sarah F. Schoch, Salome Kurth and Helene Werner published in *Journal of Sleep Research*, 2020

      This publication is a systematic review of the literature on infant actigraphy sleep research. It aims to characterize the complete-

ness of methodological reporting and issue further guidelines for actigraphy research and reporting in infants and children.

b) Standardization of analysis pipeline

Article 2: Actimetry in infant sleep research: an approach to facilitate comparability
Sarah F. Schoch, Oskar G. Jenni, Malcolm Kohler and Salome Kurth published in *SLEEP*, 2019

Here we aim to increase comparability across different research by 1) comparing two commonly used algorithms and 2) establishing an analysis pipeline that improves agreement between different algorithms.

c) Standardization of variable selection

Article 3: Which are the central aspects of infant sleep? The dynamic of sleep composites across infancy
Sarah F. Schoch, Reto Huber, Malcolm Kohler and Salome Kurth published in *Sensors*, 2020

This publication aims to standardize the variable selection process by applying a principal component analysis to find underlying sleep composites. Additionally, it uncovers findings about infant sleep across the first year of life.

2. Explore the maturation of sleep across development

The second part of my thesis focuses on sleep development from infancy to adolescence.

a) Examine the link between sleep and gut bacteria maturation in the first year of life

Articles 3 and 4 are the culmination of our large cohort study, where we followed infants across the first year of life to measure their sleep and gut bacteria objectively. We assessed infants at 3, 6, and 12 months of age, including a behavioral follow-up at 24 months.

Article 4: From Alpha Diversity to ZZZ: Exploring associations among sleep, gut bacteria and behavioral development in infancy

Sarah F. Schoch, Josue L. Castro-Meija, Lukas Krych, Witold Kot, Bingfeng Leng, Malcolm Kohler, Reto Huber, Gerhard Rogler, Luc Biedermann, Jean-Claude Walser, Dennis S. Nielsen and Salome Kurth published as preprint on *Open Science Framework*

This research article aims to illuminate the sleep-gut axis in the first year of life. Additionally, it examines if and how sleep and gut bacteria are related to behavioral development, both at the same time and prospectively.

b) Examine Spatio-temporal properties of sleep slow waves across development

We explored how the spatio-temporal properties of slow wave sleep change across development. Slow waves are a crucial marker of sleep depth and have been found to reflect neural maturation processes. Interestingly, slow waves travel across the brain and scalp spreading out from their origin. This traveling behavior can give us insight into the changes in white matter maturation across development.

i. Overnight changes in sleep slow waves across childhood and adolescence

Article 5: Across-night dynamics in traveling sleep slow waves throughout childhood
Sarah F. Schoch, Brady A. Riedner, Sean C. Deoni, Reto Huber, Monique K. LeBourgeois and Salome Kurth published in *SLEEP* in 2018

This article aims to examine traveling sleep slow waves in childhood and adolescence. Specifically, it characterizes slow waves as a traveling wave and investigates how traveling parameters (distance, speed, area) change across one night of recording.

ii. Origins of sleep slow waves in development

Research article: Spatio-temporal properties of sleep slow waves and implications for development
Igor Timofeev, **Sarah F. Schoch**, Monique K. LeBourgeois, Reto Huber, Brady A. Riedner and Salome Kurth published

in *Current Opinion in Physiology* in 2020

This article proposes standardized terminology to distinguish between intracellular and scalp recordings. Additionally, it reviews evidence on spatio-temporal properties of sleep slow waves focusing on changes across development and potential functions.

6

RESEARCH ARTICLES

6.1 ARTICLE 1

Actigraphy in sleep research with infants and young children: Current practices and future benefits of standardized reporting

Sarah F. Schoch, Salome Kurth* & Helene Werner*
*Shared last authorship

Abstract

Actigraphy is a cost-efficient method to estimate sleep–wake patterns over long periods in natural settings. However, the lack of methodological standards in actigraphy research complicates the generalization of outcomes. A rapidly growing methodological diversity is visible in the field, which increasingly necessitates the detailed reporting of methodology. We address this problem and evaluate the current state of the art and recent methodological developments in actigraphy reporting with a special focus on infants and young children. Through a systematic literature search on PubMed (keywords: sleep, actigraphy, child *, preschool, children, infant), we identified 126 recent articles (published since 2012), which were classified and evaluated for reporting of actigraphy. Results show that all studies report on the number of days/nights the actigraph was worn. Reporting was good with respect to device model, placement and sleep diary, whereas reporting was worse for epoch length, algorithm, artefact identification, data loss and definition of variables. In the studies with infants only (n = 58), the majority of articles (62.1%) reported a recording of actigraphy that was continuous across 24 hr. Of these, 23 articles (63.9%) analysed the continuous 24-hr data and merely a fifth used actigraphy to quantify daytime sleep. In comparison with an evaluation in 2012, we observed small improvements in reporting of actigraphy methodology. We propose stricter adherence to standards in reporting methodology in order to streamline actigraphy research with infants and young children, to improve comparability and to facilitate big data ventures in the sleep community.

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REVIEW PAPER

Actigraphy in sleep research with infants and young children: Current practices and future benefits of standardized reporting

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Abstract

Actigraphy is a cost-efficient method to estimate sleep–wake patterns over long periods in natural settings. However, the lack of methodological standards in actigraphy research complicates the generalization of outcomes. A rapidly growing methodological diversity is visible in the field, which increasingly necessitates the detailed reporting of methodology. We address this problem and evaluate the current state of the art and recent methodological developments in actigraphy reporting with a special focus on infants and young children. Through a systematic literature search on PubMed (keywords: sleep, actigraphy, child *, preschool, children, infant), we identified 126 recent articles (published since 2012), which were classified and evaluated for reporting of actigraphy. Results show that all studies report on the number of days/nights the actigraph was worn. Reporting was good with respect to device model, placement and sleep diary, whereas reporting was worse for epoch length, algorithm, artefact identification, data loss and definition of variables. In the studies with infants only (n = 58), the majority of articles (62.1%) reported a recording of actigraphy that was continuous across 24 hr. Of these, 23 articles (63.9%) analysed the continuous 24-hr data and merely a fifth used actigraphy to quantify daytime sleep. In comparison with an evaluation in 2012, we observed small improvements in reporting of actigraphy methodology. We propose stricter adherence to standards in reporting methodology in order to streamline actigraphy research with infants and young children, to improve comparability and to facilitate big data ventures in the sleep community.

KEYWORDS

accelerometry, actimetry, diary, guidelines, nap, rules, sensor

1 | INTRODUCTION

Actigraphy is a non-intrusive method using wristwatch-like devices to monitor movements over extended periods. The objective estimation of sleep–wake patterns from actigraphy is based on the observation that there is less movement during sleep than during wake. Validation studies have shown that sleep estimation by actigraphy correlates well with sleep scored from polysomnography (Ancoli-Israel

Salome Kurth and Helene Werner shared last authorship

[Correction added after first online publication on 20 August 2020: All mentions of the reference Meltzer, Walsh, Traylor, & Westin (2012) are incorrect. These have been amended in the text and reference list to refer to (Meltzer, Montgomery-Downs, Insana, & Walsh (2012).]

et al., 2003; Sadeh, Lavie, Scher, Tirosh, & Epstein, 1991). Actigraphy is a convenient and cost-efficient method to record sleep data over multiple days in a natural environment. This is especially useful in infants and young children, who might not sleep well in a laboratory environment (Sadeh, Alster, Urbach, & Lavie, 1989; Sadeh et al., 1991). However, a disadvantage of current actigraphy research is the variation in study procedures, including usage of different devices, and differences in data processing and analysis (Ancoli-Israel et al., 2003). Currently, there are no standardized and unique scoring rules for actigraphy, unlike Rechtschaffen and Kales gold-standard sleep scoring rules for polysomnography (Rechtschaffen & Kales, 1968). This affects outcomes and comparability across actigraphy studies (Acebo & LeBourgeois, 2006). Over the last decades, the use of actigraphy in sleep and paediatric research has significantly increased (Meltzer, Montgomery-Downs, Insana, & Walsh, 2012) as the field is undergoing rapid technological progress. The variability across actigraphy studies may further increase over the next years due to the introduction of new devices and smart phone applications. One core problematic that accompanies this technological development is that sleep variables cannot systematically be quantified in a comparable way. This has severe consequences for determining normative data and identifying clinical cut-offs for sleep problems in children. This rapidly growing methodological diversity urgently necessitates a move towards standardized reporting criteria.

Detailed information about the device, the simultaneous use of a sleep diary, data collection, data processing and sleep variables would facilitate comparability between studies (Meltzer, Montgomery-Downs, Insana, & Walsh, 2012). Standardized guidelines for defining and scoring daytime sleep are especially relevant for infants and young children (Galland, Meredith-Jones, Terrill, & Taylor, 2014), as daytime sleep accounts for up to 20% of sleep in the first year of life and persists until later childhood (at age 6 years, 5% of children are still napping) (Iglowstein, Jenni, Molinari, & Largo, 2003). Currently, either non-validated night-time rules are used for defining daytime sleep, or else actigraphy analysis is restricted to night-time data only (Galland et al., 2014). The latter is common, but it unfortunately considerably underestimates total sleep time and therefore leads to mischaracterization of infants' overall sleep . If possible and feasible, the inclusion of daytime sleep is strongly encouraged, unless the research question exclusively relates to night-time sleep (e.g., wakings in the night). Further, pronounced irregularity of sleep timing in infants and external movement due to variability of their sleep conditions (being carried/in a pushchair) and bed sharing , complicate the quantification of sleep in infants and young children. The investigation of both daytime and night-time sleep thus would benefit from standardized methodological reporting.

Specific recommendations for reporting of actigraphy were published in 2012 (Meltzer, Montgomery-Downs, et al., 2012). Since then, no follow-up review has evaluated reporting practices in the published literature. Our study summarizes the current state of the art, as well as recent methodological developments in actigraphy reporting, with a focus on infants and young children.

Practice Points

When using actigraphy

- Specify exact device (manufacturer, name, model and version)
- Devices with access to raw data and open-source software should be preferred
- Provide epoch length for both recording and analysis (if resampling is carried out)
- Report algorithm for analysis of sleep–wake variables, including a reference to the computation
- Use a diary for cross-comparison and to identify artefacts and specify whether a paper, digital or telephone diary was used
- Artifacts and missing data are sources of error or bias, for which identification and handling procedures need to be reported
- Measure and analyse actigraphy across 24 hr, especially in infants and young children
- State whether daytime sleep was taken into account or not in the calculation of total sleep time in infants and young children
- Articles with limited format or with data reanalysis nonetheless benefit from providing the above methodological anchors

2 | METHODS

2.1 | Search strategies and generation of article catalogue

Based on a systematic literature search on PubMed, we identified original English-language publications with a publication date from the year 2012 until August 15, 2019. The following keywords were used and combined with the Boolean operators "or" and "and": sleep, actigraphy, child *, preschool, children and infant. Only articles published in or since 2012, the year when the recommendations for methodological reporting of actigraphy were published (Meltzer, Montgomery-Downs, et al., 2012), were included. An overview of the number of selected articles is presented in Figure 1. In total, 447 articles were identified through the PubMed search and seven additional articles were added from a literature database generated as part of a student course (Biomedicine, University of Zurich, Switzerland). After removing duplicates, the database search yielded 454 articles. Conference abstracts, case studies, review articles, comments, study protocols and articles with non-human participants were excluded ($n = 50$). Only studies with participants with a mean age below 6.0 years, or a subgroup of participants with separately reported sleep data, were included ($n = 259$ excluded). Also, presentation of mean data from actigraphy recordings for sleep was required for inclusion ($n = 15$ excluded). Therefore, articles reporting

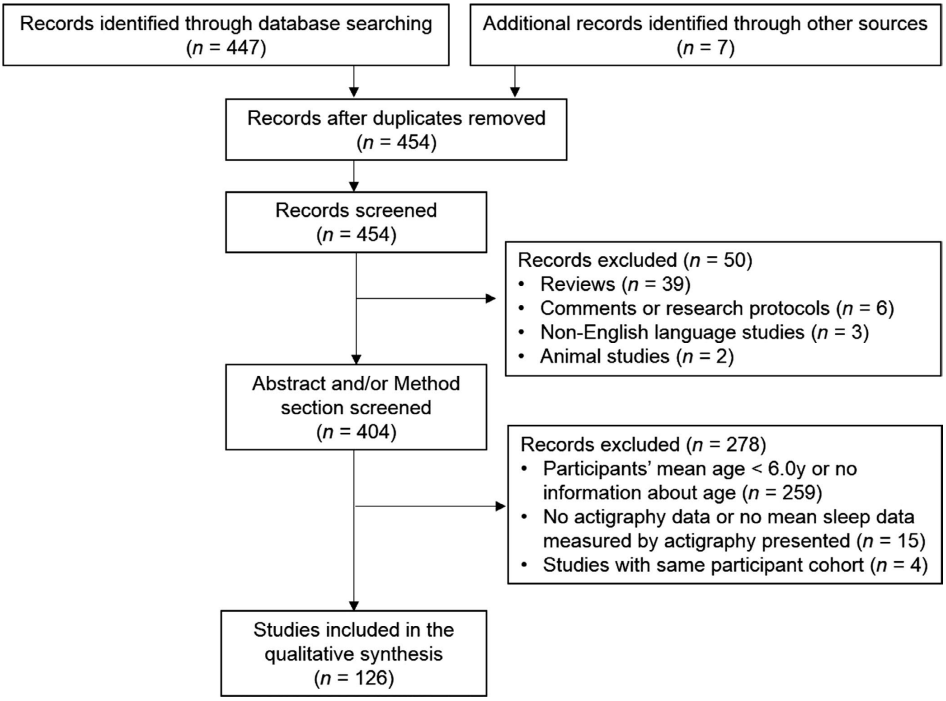

FIGURE 1 Flow chart of study participants

outcomes without further specification of sleep–wake variables or rhythmicity (i.e., only reporting sensitivity and specificity) and articles including only data regarding physical activity counts were excluded. Eight studies (four pairs) used the same participant cohort (identified by authorship, sample size, participant age and funding), of which only the first-published article from each pair was included in our analysis. This resulted in a collection of 126 articles (as marked in the reference list) that were subjected to classification according to study content and methodological quality rating.

2.2 | Article classification according to study content

All 126 articles were marked according to the description of their design and study content. First, we identified "validation studies" (including validation of device, algorithm, questionnaire, etc.). Second, we assigned the use of actigraphy-based variables to "sleep–wake variables" (e.g., sleep duration, nocturnal waking and sleep efficiency), "rhythmicity" (e.g., midsleep point, daytime sleep duration and autocorrelogram) and/or "physical activity counts" (e.g., sports activities, activity index to compute sedentary/non-sedentary, activity score). Third, we assigned the use of actigraphy data to compute "main" sleep variables or "secondary/control" measures (e.g., for confirming adherence to a specific protocol, or to identify sleep/wake in relation to other physiological measures such as body temperature, etc.). Fourth, we differentiated between inclusion of only "healthy" or also "clinical" paediatric participants with a diagnosed disorder (the latter with inclusion of a healthy control group). Prematurely born children or children with a developmental disorder, sleep problems, insomnia or night-time fears were assigned to "clinical". Fifth, age groups were assigned as "infants" (group mean age 0–12 months/0–1 year) or "young children" (13–72 m/1–5 year). Studies that investigated both infants and young children were included in the infant analysis.

2.3 | Methodological quality rating

Methodological aspects of the included studies were rated using a predefined scoring system (Table 1). This was developed in accordance with a previously published checklist (table 5 in Meltzer, Montgomery-Downs, et al., 2012). The following four overarching classifications were used, with nine items in total: (a) device and system information (four items), (b) concomitant use of a sleep diary

TABLE 1 Overview of rating criteria based on recommendations by Meltzer, Montgomery-Downs, et al. (2012)[a]

No.	Category	Rating
(a)	Device/system information	
1	Device	1 if reported, 0 if not reported
2	Placement (body part where actigraph was attached)	1 if reported, 0 if not reported
3	Epoch length (resolution with which activity counts were stored)	1 if reported, 0 if not reported
4	Algorithm	1 if a commonly used name (Cole, Sadeh, etc.) was indicated, 0 if just software name was provided but not the algorithm, or 0 also if neither the algorithm nor the software was reported (terms such as "manufacturer's algorithm" or merely reporting the sensitivity settings were counted as 0); this was not scored for articles that did not estimate sleep/wake behaviour
(b)	Sleep diary	
5	Sleep diary	1 if use of sleep diary reported, 0 if not reported
	In the articles that did report the use of a sleep diary, 3 additional criteria were rated:	
5.1	Diary type	1 if a digital sleep diary was used, 0 if not reported
5.2	Diary keeper (person completing sleep diary)	1 if reported, 0 if not reported
5.3	Diary completion frequency	1 if reported, 0 if not reported
(c)	Data collection and processing	
6	Number of days/nights	1 if reported , 0 if not reported
7	Artefact identification (methods used to identify and handle missing data or potential artifacts)	1 if reported, 0 if not reported. 1 was further assigned if the statistical approach was accounting for missing data (i.e., regression and path models using full likelihood estimation). Furthermore, 1 was also assigned if data were corrected for external motion or co-sleeping reported in a log or diary (whereas diary for measuring actigraphy removal alone was not counted)
8	Data loss	1 if data loss was reported either due to technical failure, non-adherence or artefacts
(d)	Data variables	
9	Definition of sleep variables	1 when definition of at least 1 sleep variable was provided, or a reference to the definition of particular sleep variables

Note: Points 5.1–5.3 did not count towards the total score of each article.

[a]Rating criteria are based on the proposed standard checklist for reporting actigraphy in paediatric sleep research literature presented in table 5 in Meltzer, Montgomery-Downs, et al. (2012).

during actigraphic recording (one item), (c) procedures of data collection and processing (three items), and (d) definition of sleep variables (one item). We identified information on device model and brand, as well as on device placement, epoch length, analysis algorithm, handling of artefacts, etc., for each study representing the number of fulfilled rating criteria. Sum scores ranged from 0 to 9 points, with higher scores indicating more complete reporting of methodological quality in the respective study. All three authors rated the categories for each of the 126 articles. Uncertainties were discussed among the three authors until a consensus was reached.

2.4 | Rating of daytime sleep recording in infants

Apart from the nine-item score, in the articles investigating infants, we additionally scored whether or not actigraphy was collected continuously across 24 hr (as opposed to recording intervals restricted to night or day only) and whether actigraphy was analysed across the 24-hr day. We rated articles on whether they reported on daytime sleep (e.g., naps) and whether this quantification was based on diary or actigraphy.

3 | RESULTS

The 126 articles included a total of n = 11,032 participants assessed with actigraphy. The studies contained an average of 88 participants (standard deviation [*SD*] = 111; median, 56.5; range, 5–802). Fourteen of the 126 studies (11.1%) were validation studies for either devices, algorithms or questionnaires. Most studies (94.4%) used actigraphy to assess sleep–wake patterns. Thirty-four of the 126 articles (27.0%) investigated rhythmicity and 12 (9.5%) used actigraphy to quantify physical activity (multiple categories possible; one outcome was defined in 71.4% of the articles, two in 26.2% of the articles, and three in 2.4%). One hundred and thirteen of the 126 articles (89.7%) used actigraphy as the main outcome

measure, whereas only a fraction used actigraphy as a secondary "control" variable (10.3%). Sixty-eight of the 126 articles (54.0%) investigated young children and 58 articles (46.0%) investigated infants, of which 18 articles (14.3%) investigated both infants and young children. Forty-four of the 126 articles (34.9%) included a clinical population.

3.1 | Methodological quality rating

On average, 7.4 of the nine rating criteria (*SD* = 1.4; range, 2–9) presented in Table 1 were reported per article. Most articles reported on eight (28.6%) or seven and nine rating criteria (both 23.8%). Twenty-six articles (20.7%) reported on five or six criteria and four articles (3.2%) reported on less than five rating criteria. The frequency of articles reporting each rating criterion is presented in Figure 2.

3.2 | Device/system information

One hundred and thirteen of the 126 articles (89.7%) reported the device model of the actigraph used in the respective study. These models included: Actiwatch2, AW64, Actiwatch Spectrum, Actiwatch-L and Actical (from Philips Respironics/Minimitter); Micromini Motionlogger, Motionlogger Basic, Micro Motionlogger Sleep and AMA32 (from Ambulatory Monitoring, Inc.); Actiwatch 4/Actiwatch Plus, Actiwatch mini, Motionwatch 8 and Actiheart (from Camntech); GENEactiv (from Activinsights); wActisleep-BT, GTX-BT, GT3X+, GT3X and Actisleep+ (from Actigraph); Visi Grey Flash (from Stowood); and Somnowatch (from Somnomedics). The frequency of reported actigraphy devices and brands is shown in Table 2 (for all articles and separately for articles investigating infants and young children). Twenty-one different device models were used in the 126 articles. The most common were the Actiwatch2 (Philips Respironics), which was the most frequently used model among studies with young children, and the Micromini Motionlogger (Ambulatory Monitoring, Inc.), which was the most frequently used model among studies with infants. Seven different brands were used. The brand name was reported by all articles (100%). The most common brands were Philips Respironics and Ambulatory Monitoring, Inc. Although Ambulatory Monitoring, Inc. was the most frequently used brand among studies with infants, Philips Respironics was most popular among studies with young children.

The reporting frequency of device placement, epoch length and algorithm is presented in Table 3. One hundred and nineteen of the 126 articles (94.4%) reported the device placement on the body. Both ankle/calf/leg and arm/wrist placements were common, at 38.1% and 37.3%, respectively. Ankle/calf/leg placements were most common in studies with infants and arm/wrist placements in studies with young children. Epoch length was reported in 97 of the 126 articles (77.0%). Nearly half of the studies used an epoch length of 1 min (49.2%). One hundred and eighteen of the 126 articles (93.7%) presented data on algorithm-based variables. Sixty-two of the 118 articles (52.5%) specified the algorithm used for identifying sleep and wake (by name or by providing a reference), 43 articles (36.4%) reported only the software (yet no algorithm) applied to analyse the data (including sensitivity threshold), and 13 articles (11.0%) reported neither. The most common algorithms applied for infants and young children were Sadeh, Acebo, Seifer, Aytur, and Carskadon (1995), Sadeh (1994) and Sadeh et al. (1989). A few articles analysed their data by multiple algorithms (4%).

3.3 | Sleep diary

One hundred and five of the 126 articles (83.3%) reported the use of a sleep diary. None of these articles reported the use of a digital diary. One hundred and two of the 105 articles (97.1%) reported the person who completed the diary ("diary keeper") and 72 articles (68.6%) reported how often the diary was filled out.

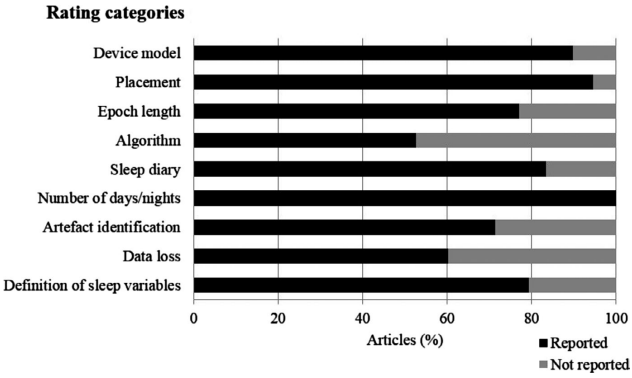

FIGURE 2 Frequencies of articles reporting predefined methodological aspects of actigraphy (n = 126 articles)

TABLE 2 Frequency of reported actigraphy devices and brands in article catalogue, presented for all articles and separately for articles investigating infants and young children

	All articles ($n = 126$), %	Articles including infants ($n = 58$), %	Articles including young children ($n = 68$), %
Device model			
Actiwatch2 (Philips Respironics)	20.6	14.0	22.7
Micromini Motionlogger (Ambulatory Monitoring, Inc.)	16.7	19.3	15.9
AW64 (Philips Respironics)	11.9	10.5	12.5
Actiwatch Spectrum (Philips Respironics)	9.5	3.5	11.4
Motionlogger Basic (Ambulatory Monitoring, Inc.)	6.3	1.8	8.0
Micro Motionlogger Sleep (Ambulatory Monitoring, Inc.)	6.3	12.3	3.4
Actiwatch 4/Actiwatch Plus (Camntech)	4.8	7.0	3.4
Actiwatch mini (Camntech)	3.2	5.3	1.1
Actical (Philips Respironics/Minimitter)	3.2	5.3	2.3
Actiwatch-L (Philips Respironics)	2.4	3.5	1.1
GENEactiv (Activinsights)	1.6	1.8	1.1
AMA32 (Ambulatory Monitoring, Inc.)	1.6	1.8	1.1
wActisleep-BT (Actigraph)	0.8	0	1.1
Visi Grey Flash (Stowood)	0.8	0	1.1
Somnowatch (Somnomedics)	0.8	0	1.1
Motionwatch 8 (Camntech)	0.8	0	1.1
GTX-BT (Actigraph)	0.8	0	1.1
GT3X+ (Actigraph)	0.8	1.8	1.1
GT3X (Actigraph)	0.8	0	1.1
Actisleep+ (Actigraph)	0.8	1.8	0
Actiheart (Camntech)	0.8	1.8	1.1
Not reported/identifiable	10.3	14.0	10.2
Brand			
Philips Respironics	48.4	36.8	52.3
Ambulatory Monitoring, Inc.	37.3	47.4	33.0
Camntech	7.9	10.5	6.8
Actigraph	4.0	3.5	4.6
Activinsights	1.6	1.8	1.1
Somnomedics	0.8	0.0	1.1
Stowood	0.8	0.0	1.1
Not reported	0.0	0.0	0.0

Note: Eight studies contained multiple devices and brands and were thus included in each respective item.

3.4 | Data collection and processing

All studies (100%) described the length of assessment duration, varying from a few hours up to 1 month. Ninety of the 126 articles (71.4%) reported the procedure for identification and correction of artefacts. Seventy-six of the 126 articles (60.3%) reported on data loss.

3.5 | Data variables

One hundred of the 126 articles (79.4%) defined at least one of the sleep variables used in the study.

3.6 | Reporting of daytime sleep in articles with infants

We next specifically focused on the reporting of actigraphy-based daytime sleep in articles that included infant participants ($n = 58$ out of the 126 articles; Figure 3). Thirty-six of the 58 studies (62.1%) recorded actigraphy across 24 hr. The remainder either recorded data during shorter intervals (e.g., from bedtime in the evening to wake-up time in the morning, 32.8%) or else it was unclear which timeframe was recorded (5.2%, "not reported" in Figure 3). Of the 36 articles that recorded continuously across 24 hr, 23 articles (63.9%) analysed 24-hr data. Twenty-one of the 58 articles (36.2%) reported

TABLE 3 Frequency of reported actigraphy device placements, epoch length and algorithm

	All articles ($n = 126$), %	Articles including infants ($n = 58$), %	Articles including young children ($n = 68$), %
Device placement			
Ankle/calf/leg	38.1	75.4	13.6
Arm/wrist	37.3	8.8	51.1
Waist/hip	3.2	0	4.5
Chest	0.8	1.8	1.1
Multiple device placements within the same study			
Ankle or wrist	12.7	8.8	17.0
Wrist or shoulder	1.5	0	2.3
Ankle or waist	0.8	1.8	1.1
Not reported	5.6	3.5	8.0
Epoch length			
< 1 s	2.4	5.3	0
15 s	7.1	8.8	5.8
30 s	17.5	19.3	17.2
1 min	49.2	49.1	50.6
2 min	0.8	1.7	0
Not reported	23.0	15.8	26.4
Algorithm			
Sadeh et al. (1995)	23.7	46.9	15.6
Sadeh (1994)	9.3	6.1	11.1
Sadeh et al. (1989)	9.3	4.0	10.0
Oakley/Respironics (1997)	9.3	2.0	11.1
Cole, Kripke, Gruen, Mullaney, and Gillin (1992)	5.1	4.1	4.4
Galland, Kennedy, Mitchell, and Taylor (2012)	3.3	6.1	2.2
Others	2.4	2.0	3.3
Not reported	47.5	36.7	46.7

Note: Five studies used multiple algorithms.

at least one variable quantifying daytime sleep by using either actigraphy (20.7%) or a sleep diary (15.5%).

3.7 | Comparison with previous methodological reporting

To derive trends in methodological reporting of actigraphy over the last few years, we compared our results with the report of Meltzer, Montgomery-Downs, et al. (2012) (Meltzer, Montgomery-Downs, et al., 2012). Comparison of reported brands, epoch length, device placement and algorithm between the study by Meltzer, Walsh, et al. (2012) and our study is presented in Table 4. Since the evaluation of 2012, the same device brands are predominantly used. Yet, our study revealed less use of Ambulatory Monitoring, Inc. and Camntech, whereas Philips Respironics was more frequently used. All articles reported the brand in our study, whereas a small number of studies did not report it in the evaluation by Meltzer, Montgomery-Downs, et al. (2012). Although in Meltzer, Montgomery-Downs, et al. (2012) a predominance of device placement on the wrist/arm was obvious, we also found common placement on the ankle, calf or leg. Furthermore, whereas Meltzer, Montgomery-Downs, et al. (2012) only included the wrist/arm and ankle/calf/leg, our analysis revealed frequent reporting of other device placements, such as on the shoulder and multiple device placements. Among various possible epoch lengths, the use of 1 min is still the most frequent, yet with a tendency towards the use of finer resolutions. Also, the lack of reporting epoch length was less frequent in our study compared to in the evaluation by Meltzer, Montgomery-Downs, et al. (2012). Compared to the study by Meltzer, Montgomery-Downs, et al. (2012), fewer

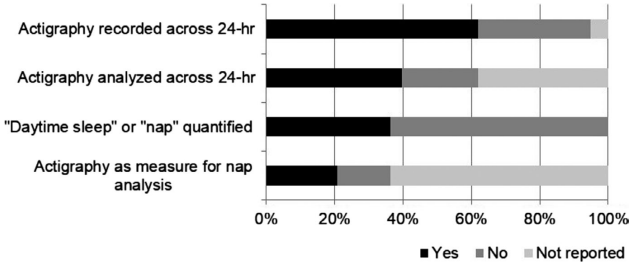

FIGURE 3 Frequencies of reported daytime sleep in articles investigating infant participants ($n = 58$ articles)

articles used the Sadeh algorithm and more articles did not report the algorithm in our study.

4 | DISCUSSION

We performed a comprehensive analysis of recent actigraphy research in infants and young children to identify developments in actigraphy methodology for sleep research. Through an extensive and systematic literature search, we identified 126 articles, which were evaluated for reporting of actigraphic methodology based on evidence-based recommendations (Meltzer, Montgomery-Downs, et al., 2012). Reporting on the number of days/nights the actigraph was worn was excellent, as it was indicated in all articles. Reporting was good (i.e., >80%) with respect to device, placement and sleep diary. Reporting for epoch length, algorithm, artifact identification, data loss and definition of variables was worse and would benefit from improvement in the future.

Our study shows that all articles reported the actigraphy brand, whereas 10.3% of the articles did not report the specific device model used. Two actigraphy brands are dominant among infants and young children (Philips Respironics and Ambulatory Monitoring). Introduction of new models by manufacturers and the fact that certain devices are no longer developed and some companies have merged (e.g., Mini Mitter with Philips Respironics) can lead to changes in data collection and induce a source of error. Thus, it is important to comprehensively report device information in future studies. Nearly half of the studies (47.5%) did not report on the analysis algorithm used for sleep variable computation, with 32.7% providing partial information (refer to the software/threshold applied, yet lack information on the algorithm itself) and 12.9% providing no information about the algorithm. This is problematic because estimates of sleep–wake variables greatly depend on algorithms, particularly in infants (Schoch, Jenni, Kohler, & Kurth, 2019), and ambiguity arises when the software offers multiple options based on different algorithms or different versions of software might use different algorithms. Algorithm changes in commercial software are generally possible, even without the awareness of users. Furthermore, not every algorithm has been validated in each device for every age group. Consequently, the choice of algorithm depends on the device. Thus, detailed reporting of the algorithm and specific adjustments to the computation are crucial. However, in the case when analysis software is proprietary (and therefore the computational source code is not available), researchers cannot obtain information on the applied algorithm. Additionally, not every software has implemented each algorithm. Thus, the users might be limited in which algorithms they can effectively apply. Nonetheless, providing detailed information is crucial and it should ideally include the analytical formula or reference to an article with the formula. An urgently needed future direction is the cross-comparison of sleep variable outcomes among the existing algorithms and software. Only this advance will allow proper comparison of objective sleep measures in big data ventures and will lead to discoveries of sleep differences among culture, geography, age, health status, etc.

Only 60% of the articles provided information on data loss, which can mainly result from technical failure or participants' non-adherence. However, more details on reasons underlying data loss would be welcomed because it provides essential information about the dropouts from the study and about possible bias of the results. Among the 126 articles, an epoch length of 1 min was most commonly used. The selection of epoch length largely depends on the study design and research question (e.g., high-resolution data sampling for quantification of micro arousals versus longitudinal data across a year). Furthermore, more recent devices offer more setting options for sampling data with higher time resolution. Nonetheless, nearly one-quarter of articles did not report epoch length.

Nearly a fifth of the studies did not use or did not report the use of a sleep diary. Diary use depends on the research question and design of the study, and particularly in polysomnography studies diary use might be less important. However, the inclusion of a diary improves the handling of data loss and artifacts and is preferred to using data without a diary (Tetreault, Belanger, Bernier, & Carrier, 2018). In our study, we also tried to xml:identify which type of diary (paper versus digital versus smartphone) was used. However, this information was frequently unclear. Most studies use a paper diary, which was not explicitly specified in the articles. Considering that novel trends may include more digitized diaries in the future, specifics on diary type (also the paper diary) should be given.

Even though the majority of infant studies measured actigraphy continuously across 24 hr (62.1%), only 64% of these analysed complete 24-hr intervals. This reveals that large parts of the recording are often discarded, even though they contain valuable information about infants' sleep. Furthermore, only 12 of the 58 articles (20.7%) used actigraphy to analyse daytime sleep, a small number given that daytime

TABLE 4 Frequency of reported brands, epoch length, device placement and algorithm: comparison with Meltzer, Montgomery-Downs, et al. (2012)

	Meltzer, Montgomery-Downs, et al. (2012)[a] %	Current rating, %
Brand		
Philips Respironics (previously Mini-Mitter)	21.7	48.4
Ambulatory Monitoring, Inc.	56.0	37.3
Camntech (previously Cambridge Actiwatch)	11.4	7.9
Other brands	4.8	7.2
Not reported	6.0	0.0
Device placement[b]		
Ankle/calf/leg	22.9	38.1
Arm/wrist	67.5	37.3
Other/multiple	0.0	19.0
Not reported	9.0	5.6
Epoch length [c]		
15 s	1.6	7.1
30 s	3.9	17.5
1 min	62.2	49.2
Other	0.0	3.2
Not reported	32.2	23.0
Algorithm		
Sadeh [d]	65.6	40.8
Oakley/Respironics [e]	0.0	7.9
Cole-Kripke	6.5	2.4
University of California San Diego	1.1	0.0
Other	0.0	4.8
Not reported	26.9	44.1

[a]Limited sample with $n = 166$ was taken for comparison; see second column in table 5 in the publication by Meltzer, Montgomery-Downs, et al. (2012).

[b]'Arm/wrist' in the publication by Meltzer et al. was defined by the sum of the frequency reported for 'non-dominant wrist', 'dominant wrist' and 'wrist unspecified'. Only studies with wrist and ankle actigraphy included.

[c]Due to the fact that the epoch length in Meltzer, Montgomery-Downs, et al. (2012) was presented separately for Ambulatory Monitoring, Inc. and Mini-Mitter, its frequency was combined for comparison.

[d]The different versions of the Sadeh algorithms were summarized.

[e]Meltzer, Montgomery-Downs, et al. (2012) included algorithms with the Ambulatory Monitoring, Inc, but not with Mini-Mitter devices, which commonly use the Respironics/Oakley algorithm.

sleep accounts for up to 20% of sleep in the first year of life (Iglowstein et al., 2003). Thus, in future studies, it should be clearly stated whether daytime sleep was considered or not in the calculation of total sleep time in infants and young children. In addition, we believe that the research field would benefit from evaluation of daytime sleep and the description of this procedure in as much detail as possible. Current knowledge on sleep regulation indicates that daytime sleep influences night-time sleep, not only in adults (Campbell & Feinberg, 2005), but also young children (Lassonde et al., 2016). Although daytime sleep can be more complex to assess and quantify (involvement of daycare/nannies, external movements during sleep, etc.), we nonetheless strongly recommend the inclusion of daytime sleep if feasible.

Compared to the evaluation by Meltzer *et al.* in 2012 (Meltzer, Montgomery-Downs, et al., 2012), we observed the following recent developments: device, brand, device placement and epoch length reporting have improved over time, although are still not complete. Specifically, we observed increased reporting of the device, although model specification was still lacking in 10.3% of the cases. We also observed a change in popularity of devices: Ambulatory Monitoring, Inc. has been replaced by Philips Respironics. This may be due to costs or availability of validation reports for the device, or due to the fact that a broader age range was included in the study by Meltzer, Montgomery-Downs, et al. (2012) than in our study (0–18 years versus 0–6 years). Additionally, new brands have been introduced in the field. This development is not surprising considering the rapid market growth of wearables. As more commercial devices become available, it is important to keep in mind that these may not be suitable for research with infants and young children, unless validation and replication are provided in the specific age group. Furthermore, a number of devices have analysis algorithms that are not publicly available, which also includes the problem that algorithms are sometimes changed without notice to the user. Another interesting observation was that reporting of device placement increased slightly. It seems to have become more common to allow flexibility with device placements. This might be caused by the investigation of different cohorts, heightened non-compliance of participants with certain placements, studies indicating no differences regarding device placement, or physical activity researchers using waist/hip placements entering into sleep research (which were not looked at in the study by Meltzer, Montgomery-Downs, et al., 2012). For example, in young children, sleep–wake estimates from the ankle and wrist were reported to be comparable (Belanger, Bernier, Paquet, Simard, & Carrier, 2013).

It needs to be mentioned that articles published in 2012 may be under-represented in the analysis by Meltzer et al. and in the current report; however, we only wanted to include articles after publication of the previous article. Furthermore, articles referring exclusively to "accelerometry" were possibly missed.

Non-standardized computation of sleep variables across studies can cause variation in study outcomes. Similarly, study design, device parameters and analysis choices add variability and ultimately prevent cross-comparison among studies. Even though journals limit the length of research articles, there is a need for minimal actigraphy methodological anchors. Although details on actigraphy methodology may be less important in a review article or comment, they are essential to improve reproducibility in articles presenting original data. As daytime sleep can account for up to 20% of total sleep duration in infants and young children, it is crucial that daytime sleep is not neglected in research including infants and young children. The analysis

of daytime sleep is complicated by the variability of sleep conditions with added external movement (e.g., being carried/in pushchair). However, information recorded in the sleep diary supports the addressing of such artefacts and facilitates the identification of daytime sleep (Schoch et al., 2019). Validation of existing algorithms for use with naps without reliance on diary reports will be necessary in the future if naps are to be analysed in large-scale datasets.

We believe that combining actigraphic data from multiple cohorts has large potential for "big data" approaches. Analyses of large datasets have higher statistical power and can provide novel insights (e.g., regarding cultural variability in the development of sleep rhythms). However, pooling data requires detailed reporting for each individual dataset. Validation of each device on the market, including the application of different algorithms as well as specificity for age groups, and direct comparative analysis across devices would support the development of a "gold standard" for actigraphy data analysis. Because we anticipate continued future developments, a database to collect such information on devices would be useful and could be integrated with a database including available datasets.

5 | CONCLUSIONS

There is ongoing rapid growth of methodological diversity in actigraphy-based sleep research. This diversity causes large differences in sleep outcome variables (Schoch et al., 2019), which complicates translation to clinical practice. Heterogeneity of sleep monitoring with actigraphy has diverse causes, most of which can be overcome through stricter adherence to methodological standards. Standardized reporting of methodological details should be maintained or improved in the future. The community would benefit from more approaches that directly compare sleep outcomes with different methodologies (Schoch et al., 2019). Comparability across disciplines will facilitate the consolidation of existing data from wearables. Additionally, the integration of daytime sleep will support the understanding of normative sleep, particularly in infants. Providing comprehensive methodological details is a prerequisite for future public data-sharing platforms, which will advance progress in the research of sleep in infants and young children.

Research Agenda

- Our study should be extended to school-age children and adolescents in order to compare reporting practices between the age groups
- Analysis of daytime sleep is confounded by the variability of sleep conditions (e.g., external movement), yet integrating daytime sleep supports the thorough investigation of infants and young children
- The existing guidelines may be supplemented by standardized reporting criteria for defining and scoring daytime sleep in infants and young children

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CONFLICT OF INTERESTS

The authors have indicated no financial conflict of interests.

AUTHOR CONTRIBUTIONS

All authors were involved in the design, literature research, data analysis and writing of the manuscript.

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6.2 ARTICLE 2

Actimetry in infant sleep research: an approach to facilitate comparability

Sarah F. Schoch, Oskar G. Jenni, Malcolm Kohler & Salome Kurth

Abstract Study Objectives: Only standardized objective assessments reliably capture the large variability of sleep behavior in infancy, which is the most pronounced throughout the human lifespan. This is important for clinical practice as well as basic research. Actimetry is a cost-efficient method to objectively estimate infant sleep/wake behavior from limb movements. Nevertheless, the standardization of actimetry-based sleep/wake measures is limited by two factors: the use of different computational approaches and the bias towards measuring only nighttime sleep—neglecting 20 % of sleep infants obtain during daytime. Thus, we evaluate the comparability of two commonly used actimetry algorithms in infants and propose adjustments to increase comparability. Methods: We used actimetry in 50 infants for 10 continuous days at ages 3, 6, and 12 months in a longitudinal approach. We analyzed the infants' sleep/wake behaviors by applying two algorithms: Sadeh and Oakley/Respironics. We compared minute-by-minute agreement and Kappa between the two algorithms, as well as the algorithms with sleep/wake measures from a comprehensive 24-hour parent-reported diary. Results: Agreement between uncorrected algorithms was moderate (77%–84%). By introducing a six-step adjustment, we increased agreement between algorithms (96%–97%) and with the diary. This decreased the difference in estimated sleep behaviors, e.g. Total Sleep Duration from 4.5 to 0.2 hours. Conclusions: These adjustments enhance comparability between infant actimetry studies and the inclusion of parent-reported diaries allows the integration of daytime sleep. Objectively assessed infant sleep that is comparable across different studies supports the establishment of normative developmental trajectories and clinical cutoffs.

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Actimetry in infant sleep research: an approach to facilitate comparability

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Abstract

Study Objectives: Only standardized objective assessments reliably capture the large variability of sleep behavior in infancy, which is the most pronounced throughout the human lifespan. This is important for clinical practice as well as basic research. Actimetry is a cost-efficient method to objectively estimate infant sleep/wake behavior from limb movements. Nevertheless, the standardization of actimetry-based sleep/wake measures is limited by two factors: the use of different computational approaches and the bias towards measuring only nighttime sleep—neglecting ~20 % of sleep infants obtain during daytime. Thus, we evaluate the comparability of two commonly used actimetry algorithms in infants and propose adjustments to increase comparability.

Methods: We used actimetry in 50 infants for 10 continuous days at ages 3, 6, and 12 months in a longitudinal approach. We analyzed the infants' sleep/wake behaviors by applying two algorithms: Sadeh and Oakley/Respironics. We compared minute-by-minute agreement and Kappa between the two algorithms, as well as the algorithms with sleep/wake measures from a comprehensive 24-hour parent-reported diary.

Results: Agreement between uncorrected algorithms was moderate (77%–84%). By introducing a six-step adjustment, we increased agreement between algorithms (96%–97%) and with the diary. This decreased the difference in estimated sleep behaviors, e.g. *Total Sleep Duration* from 4.5 to 0.2 hours.

Conclusions: These adjustments enhance comparability between infant actimetry studies and the inclusion of parent-reported diaries allows the integration of daytime sleep. Objectively assessed infant sleep that is comparable across different studies supports the establishment of normative developmental trajectories and clinical cutoffs.

Statement of Significance

Actigraphy is a cost-efficient method to estimate sleep/wake behavior from movement. However, generalization of findings in infant sleep research has been limited due to the use of different algorithms for sleep/wake quantification and the primary focus on nighttime sleep. We optimized sleep quantification from actimetry in infants by applying a set of adjustments that overcomes discrepancies between existing algorithms in sleep estimates. This method improves analysis of daytime sleep and leads to increased comparability between studies. In the future, the inclusion of more sensors and a digital diary could lead to development of normative trajectories and enhanced clinical cutoffs.

Key words: actigraphy; pediatrics – infants; scoring; algorithm; actimetry; sleep-wake bias; sleep detection improvement

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Introduction

Studying the relationship between sleep in early life and later health and behavioral outcomes requires objective and reliable quantitative data. Current practice often relies on parent-reported questionnaires to estimate infants sleep/wake behavior. However, these parent-reported questionnaires are subjective and often disagree with more objective sleep measures, including comprehensive diaries completed by parents across consecutive 24-hour periods, e.g. misjudging sleep duration by >1 hour in young children [1]. Wearables estimate sleep/wake states from arm or leg movement (actimetry) and allow cost-efficient sleep tracking in diverse environments and over long periods of time [2]. Standardized procedures in actimetry studies will facilitate generalization of findings and cross-comparison between studies. Yet, we have to overcome two existing constraints: first, there are no standards for scoring sleep/wake from actimetry. In fact, the comparability of widely used analysis algorithms has not been investigated [3]. Second, it is important to investigate both day- and nighttime sleep in infants as sleep pressure and quality largely depend on the preceding history of day-/nighttime sleep [4]. Certain limitations (e.g. the underestimation of sleep due to external movements from carriage, stroller or bed-sharing, and the overestimation of sleep when immobilized, e.g. baby sling, breastfeeding) have confined most infant actimetry assessments to nocturnal sleep, missing the ~20% of daytime sleep [5].

This study evaluates the comparability of commonly used actimetry methodologies in infants. We compare two approaches and compute their bias to sleep or wake. We then propose adaptations to streamline sleep/wake identification and to quantify infant daytime sleep by integrating 24-hour diary information into the analysis [6]. Increased comparability across actimetry-based sleep estimates reduces sources of variability for ultimately framing sleep–wake patterns and normative sleep in infants.

Methods

Participants

Fifty healthy term-born infants (17 females) were longitudinally assessed with ankle actimetry at age 3 months (i.e. 2.46–3.38 months at assessment start), 6 months (5.42–6.18 months), and 12 months (11.47–12.16 months). The presence of medical conditions (e.g. diseases or lesions of the central nervous system, developmental disabilities, epilepsy, neurologic/metabolic disorders/head injury involving loss of consciousness) and travelling across time zones with >1 hour difference in the 4 weeks prior to assessment served as exclusion criteria. Ethical approval was obtained from the Zurich Ethics Committee (2016-00730) and study procedures were consistent with the declaration of Helsinki. Written parental consent was obtained before enrollment.

Experimental design

Data were collected at each assessment time point for a duration of 10 days (5–16 days) through ankle actimetry and a 24-hour sleep–wake diary. GENEactiv accelerometers (Activinsights Ltd, Kimbolton, UK; 43 × 40 × 13 mm, Micro-Electro-Mechanical Systems sensor, 16 g, 30 Hz frequency; sensitive for ±8 g range at 3.9 mg resolution) were attached on the left ankle with a modified sock or a Tyvek paper strap. Parents were instructed to only remove the actimeter for bathing and to document its removal in the 24-hour diary. The sleep diary was adapted from Werner et al. [1], with parents reporting in 15-minute intervals: sleep (including external movement, e.g. sleeping in the parents arms, stroller *etc.*), wake, feeding, and crying. Parents reported bed times (putting infant to bed in the evening and getting up in the morning) and naps, and marked particular periods of uncertainty (e.g. feeding periods during nighttime). They were instructed to fill out the diary throughout the day. During the assessment, the Brief Infant Sleep Questionnaire (BISQ) was completed [7]. Families received small gifts for the infant (i.e. bottles, baby food) for participation.

Actimetry processing

Actimetric data were extracted as binary files using GENEactiv PC Software (version 3.1), imported into Matlab (R2016b), and converted to activity counts [8], including a 3–11 Hz bandpass filter and signal compression to 15-second bins. Acceleration data from the three axes were combined using a sum of squares. Signal was compressed to one data point per minute by data summation. To identify infant sleep and wake periods, several adjustments were introduced to existing algorithms (Sadeh et al. [9] and Oakley [10]; Figure 1, A). We focused on the most commonly used algorithm in the pediatric literature (Sadeh algorithm as identified in Meltzer et al. [3]) and the most commonly used device (Oakley algorithm used with Respironics devices). We implemented adjustments to algorithms that were based on frequently applied procedures (threshold, rescoring, smoothing [11–13]). An additional adjustment was used to counteract a bias towards overemphasizing sleep or wake. The order of adjustments was tailored to first adapt the algorithm (threshold and bias factor) and then adapt the scoring of sleep and wake (corrections based on sleep diary, Webster rescoring [14], smoothing). Further, the order of adjustments was chosen to prevent that adjustments interact (e.g. first the correction for external movement was completed, then smoothing was applied).

The following adjustments were performed: The first adjustment include the change of threshold. For the Oakley algorithm, this refers to the identification of the value serving as a threshold to distinguish sleep from wake. While generally a threshold of 20, 40, or 80 is used, we replaced the threshold with mean activity of the full recording*0.888 (similar to the auto-threshold setting). This threshold has been shown to work best in infants older than 2 months [11]. In contrast, the Sadeh algorithm applies a threshold to distinguish between low- and high-activity epochs. This threshold is originally set 100, which we replaced consequently with mean activity of the full recording*0.888. The second adjustment was the introduction of factor based on mean activity of each recording [12], with the aim of counteracting the strong bias to either sleep or wake of each algorithm. This factor was added (Sadeh)/subtracted (Oakley) from the computed activity (Figure 1, B). The third adjustment included the replacement of periods when the actimeter was not worn. These periods were identified with the parent-reported 24-hour diary (Figure 1, C). This adjustment step counteracted incorrect scoring of sleep originating from a lack of activity. The fourth

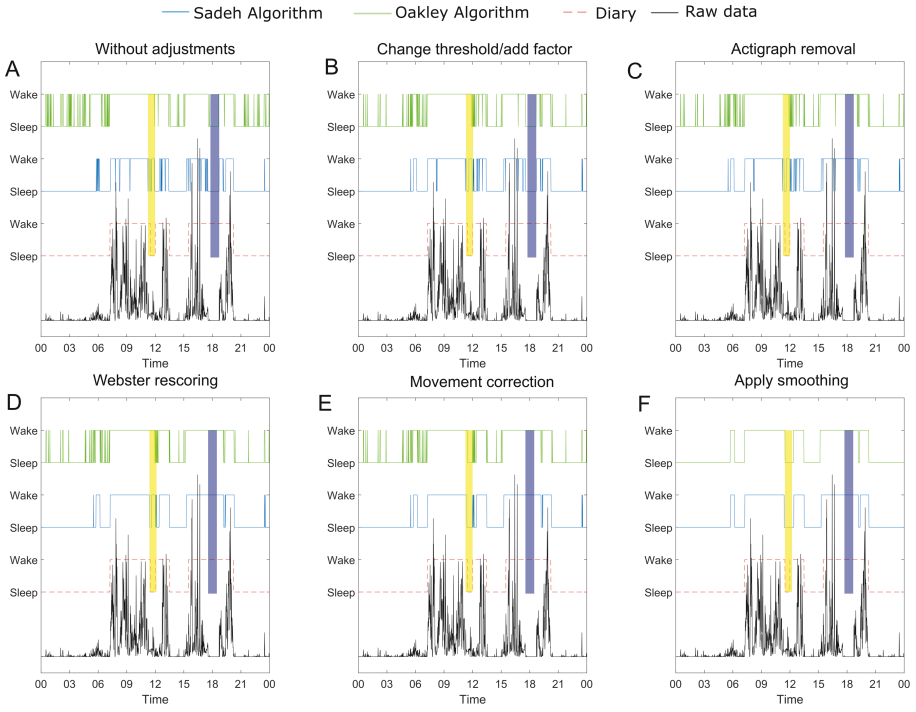

Figure 1. Stepwise processing adjustments. Typical 24-hour actimetric profile from a representative participant (age 12 months). Raw data (black) and the scorings from 24-hour diary (red), Sadeh (blue), and Oakley (green) are presented. Wake is shown on top and sleep at the bottom of each scoring item. Stepwise adjustments are presented in order of processing: (A) raw data without adjustments; (B) altered threshold and added factor reducing wake/sleep bias; (C) rescoring of actimeter removal with 24-hour diary information; (D) rescoring by Webster; (E) rescoring of sleep with external movements; and (F) smoothing of short wake periods (<5 minutes) during sleep. Yellow shading indicates periods of sleep with reported external movement. Blue shading illustrates periods with actimeter removal.

adjustment was implemented to rescore data using the strict criteria by Webster et al. [14]. This corrects mis-scoring of sleep by addressing short periods of inactivity during wake (short periods [6–10 minutes] of sleep surrounded by periods of wake and the first 1–4 minutes of sleep are rescored wake, Figure 1, D). The fifth adjustment was the correction of sleep periods with known external movement, as verified with the diary (Figure 1, E). Finally, the sixth adjustment included data smoothing. Wake periods shorter than 5 minutes were removed if they were surrounded by sleep periods. Because infants generally show higher movement activity during sleep (twitches) than older children [15, 16], smoothing ensures that sleep periods with movement are still scored as sleep (Figure 1, F).

In order to reduce error caused by external factors, 24-hour days were excluded for the calculation of sleep variables if (1) the actimeter was removed for >3 hours (in 22.2% of all data including first and last days of the assessment, and interruptions due to sickness), (2) the infant was sick but the overall assessment was continued (4.2%), or (3) the assessment took place during the switch to/from daylight savings (0.2%). These criteria resulted in the following data included in final analysis: mean assessment duration of 8.6 ± 1.65 days at age 3 months (whereby 3 days was the minimum assessment duration and 13 days, the maximum), correspondingly 8.0 ± 1.95 days included at 6 months (2–11 days), and 7.9 ± 1.71 days included at 12 months (3–10 days).

From the resulting matrix containing a minute-by-minute scoring of either sleep or wake, sleep variables of interest were computed: *Total Sleep Duration*, *Day-to-Day Sleep Variability*, *% Night Sleep*, and *Fragmentation*. *Total Sleep Duration* (hour) sums the time scored as sleep within 24 hours (starting at clock time 0:01). *Day-to-Day Sleep Variability* (hour) is the *SD* of the *Total Sleep Duration* across all included assessment days. *% Night Sleep* indicates the relative proportion of nighttime sleep (i.e. within clock time 19:00–07:00) as a percentage of *Total Sleep Duration*. *Fragmentation* (awakenings/hour) calculates the number of awakenings per hour during nighttime sleep (based on individual infant bedtimes reported by parents). Awakenings were scored separate when divided by at least 10 minutes of sleep. BISQ total sleep duration was calculated by adding reported day and night sleep duration (rounded to 15 minutes; mean was used when time range was reported). Nine BISQ assessments

were excluded due to incomplete data. To calculate the agreement between actigraphy and the 24-hour diary, the diary was transformed to a minute-by-minute scoring resolution. Feeding periods during sleep were scored as wake.

Statistical analysis

We used R (version 3.5.0) and R Studio (version 1.1.463) for statistical analyses. Linear mixed-effect models were estimated using restricted maximum likelihood to analyze changes resulting from adjustments using the R-packages *lmer* [17] and *lmertest* [18]. The covariate assessment time point was included as a logarithmic function of age (log[age]). We chose this logarithmic function to account for the flattening of effects with age (larger effects between 3 and 6 months than between 6 and 12 months). All models included effects of adjustment, infant age, and their interaction. To compare whether random effects of time point and adjustments improve model fit, we compared one model combining both random effects with two separate models containing random effects of either time point or adjustment. The random effects were only included in the final model if it significantly improved the model fit with most weight given to the Bayesian Information Criterion (BIC; Supplementary Tables 1–5, selected model highlighted in bold).

We calculated agreement between two measures as percentage of 1-minute periods scoring the same state (i.e. sleep or wake) and additionally using Cohen's Kappa [19]. Bias was calculated as the difference (minute) where one algorithm scored sleep and the other wake. We used Bland–Altman statistics to investigate whether the algorithms calculated similar estimates for sleep variables (package *BlandAltmanLeh*). Further, we tested the stability across age by investigating whether large differences between algorithms at age 3 months are also associated with large differences at older age. Accordingly, we performed correlations between time points with the difference measure resulting from the Bland–Altman statistics. A two-sided significance level of $p < 0.05$ was used.

Results

Agreement between algorithms (Sadeh algorithm–Oakley algorithm)

We compared the agreement between the algorithms with and without adjustments. Without adjustment, algorithms show moderate agreement in scoring sleep or wake (77%–84%, κ = 0.50–0.68; Table 1). Agreement was significantly improved by introducing the six-step adjustment (96%–97%, κ = 0.91–0.95, $t_{(274.63)} = 23.35$, $p < 0.0001$; Supplementary Table 1). The largest disagreement was observed in actimetry data from infants aged 3 months ($t_{(247)} = 14.44$, $p < 0.0001$). The largest improvement in agreement occurred at age 3 months (interaction age * improvements, $t_{(247)} = -7.63$, $p < 0.0001$). The improved agreement mainly results from threshold adaptation and adding the factor against bias (~5%) as well as smoothing (~2%).

Agreement between algorithms and 24-hour diary (Sadeh algorithm–24-hour-diary, Oakley algorithm–24-hour-diary)

We compared the scorings of both algorithms, with and without adjustments, with the parental-reported 24-hour diary. Both algorithms showed medium agreement with the 24-hour diary without adjustments (75%–85%, Sadeh vs Diary κ = 0.51–0.70, Oakley vs Diary κ = 0.5–0.68; Table 1). Adjustments increased agreement to up to 93% (86%–93%, Sadeh vs Diary κ = 0.72–0.86, Oakley vs Diary κ = 0.71–0.86, $t_{(494.42)} = 13.93$, $p < 0.0001$; Supplementary Table 2). Lower agreement was seen for 3-month olds compared to 6- and 12-month olds ($t_{(495)} = 12.19$, $p < 0.0001$). The observed interaction between age and improvement ($t_{(495)} = -3.31$, $p = 0.001$) indicates that adjustments lead to greater improvements particularly in the youngest age group. There was no significant effect of algorithm (Sadeh vs Oakley $t_{(1,495)} = 1.90$, $p = 0.06$) and no interaction of type of algorithm and age ($t_{(495)} = -0.32$, $p = 0.75$). A small interaction was observed between algorithm and amount of improvements, with the Oakley algorithm showing increased improvements due to the adjustments ($t_{(495)} = -2.02$, $p = 0.04$). At 3 months, adjusting for movement during sleep greatly improved the agreement (~4.5%), which was less pronounced for 6 and 12 months, respectively (~1.5%–3 %). The opposite was seen for adjustments for actimeter removal, which occurred less at 3 months (0.68%) than at 6 and 12 months (~1.25%–2%).

Bias towards sleep or wake (Sadeh algorithm–Oakley algorithm, algorithms–24-hour-diary)

Each algorithm had a scoring bias for a specific state: 200–300 minutes per day were scored as sleep by the Sadeh algorithm and wake

Table 1. Agreement rates with and without adjustment steps

Age	No adjustments	Change threshold	Add factor	Actigraph removal	Rescoring Webster	External movements	Smoothing
Sadeh–Oakley							
3 months	77.36 ± 3.97	83.21 ± 3.75	91.56 ± 1.59	91.64 ± 1.59	92.96 ± 1.34	93.56 ± 1.38	95.76 ± 1.00
6 months	83.65 ± 3.67	87.65 ± 3.68	93.14 ± 1.34	93.23 ± 1.33	93.43 ± 1.13	94.89 ± 1.16	96.79 ± 0.80
12 months	83.97 ± 3.07	87.99 ± 3.12	93.66 ± 1.32	93.78 ± 1.32	94.66 ± 1.34	94.88 ± 1.36	97.43 ± 0.83
Sadeh–Diary							
3 months	76.39 ± 6.05	75.25 ± 6.11	79.55 ± 5.19	80.22 ± 5.16	81.65 ± 5.17	86.22 ± 5.17	86.36 ± 5.20
6 months	82.26 ± 4.93	81.18 ± 4.92	84.42 ± 3.59	85.67 ± 3.60	86.86 ± 3.59	89.60 ± 3.21	89.69 ± 3.26
12 months	85.40 ± 6.23	84.30 ± 6.12	88.19 ± 5.11	90.21 ± 2.87	91.55 ± 2.66	93.07 ± 2.42	93.23 ± 2.44
Oakley–Diary							
3 months	75.14 ± 4.57	77.17 ± 4.83	77.22 ± 4.86	77.91 ± 4.81	79.24 ± 7.40	84.05 ± 4.95	85.73 ± 5.08
6 months	80.77 ± 3.34	82.77 ± 3.53	82.77 ± 3.55	84.00 ± 3.56	84.76 ± 3.41	87.85 ± 3.21	89.45 ± 3.25
12 months	84.15 ± 5.04	86.34 ± 5.11	86.36 ± 5.13	88.38 ± 2.83	89.08 ± 2.86	90.76 ± 2.72	92.99 ± 2.50

Agreement rates as % agreement (averaged over participants over measurement days). Agreements are shown between the two algorithms and between each algorithm and sleep diaries filled out by the parents. Means ± *SD* is shown.

by the Oakley algorithm (Figure 2). This bias was significantly reduced by the adjustments ($t_{(282.49)} = -27.34, p < 0.0001$; Supplementary Table 3). Particularly 3-month-olds' scorings showed increased bias in comparison with the older infants' scorings ($t_{(247)} = -13.35$, $p < 0.0001$), but bias decreased most through our adjustments at that age ($t_{(1,247)} = 11.45$, $p < 0.0001$). Similar bias was observed when compared to the 24-hour diary: the Sadeh algorithm scored more sleep than reported in the 24-hour diary. This bias decreased through the adjustments ($t_{(198)} = -6.38$, $p < 0.0001$). The bias was stronger with lower age ($t_{(100.58)} = -2.79$, $p = 0.006$) but showed no interaction with age ($t_{(198)} = 1.50$, $p = 0.13$). The Oakley algorithm scored more wake compared to the sleep 24-hour diary. This bias was significantly reduced by our adjustments ($t_{(81.81)} = 11.68$, $p < 0.0001$). There was an age effect ($t_{(90.08)} = 3.50$, $p = 0.0007$), with the largest improvements at 3 months ($t_{(198)} = -5.75$, $p < 0.0001$).

Sleep/wake behavior estimation (Sadeh algorithm–Oakley algorithm)

To estimate differences in the sleep parameters, we calculated Bland–Altman statistics of each parameter without and with adjustments (Table 2 and Figures 3–6). Without adjustments, there was a bias in the variables *Sleep Duration*, *% Night Sleep*, and *Fragmentation*, as shown by data points instead of being centered around 0 (no bias), they were centered around, e.g., 4.2 hours for *Sleep Duration* (Figure 3). Bias for each age group is shown in Table 2, e.g. 5.45 hours at age 3 months (previously this approach was used with a bias definition exceeding ± 0.5 hour) [1]. This bias was reduced by our adjustments to, *e.g.*, –0.01 hour in *Sleep Duration* at 3 months. The only variable showing low bias (mean < 0.5 hour) already without adjustments was *Day-to-Day Sleep Variability*. Taken together, we show that infant actimetry-based detection of sleep/wake variables can be improved by six-steps of adjustments.

We then tested whether differences between algorithms were stable across age. We analyzed whether differences between algorithms in sleep variable estimation were correlated between time points (i.e., 3 vs 6 resp. 12 months, Table 3). Thereby, strong positive correlations indicate a low age-effect, and for instance reveal that large differences in sleep estimates at age 3 months also show large differences at age 6 months. Generally, correlations were stronger for unadjusted data, but did not reach statistical significance.

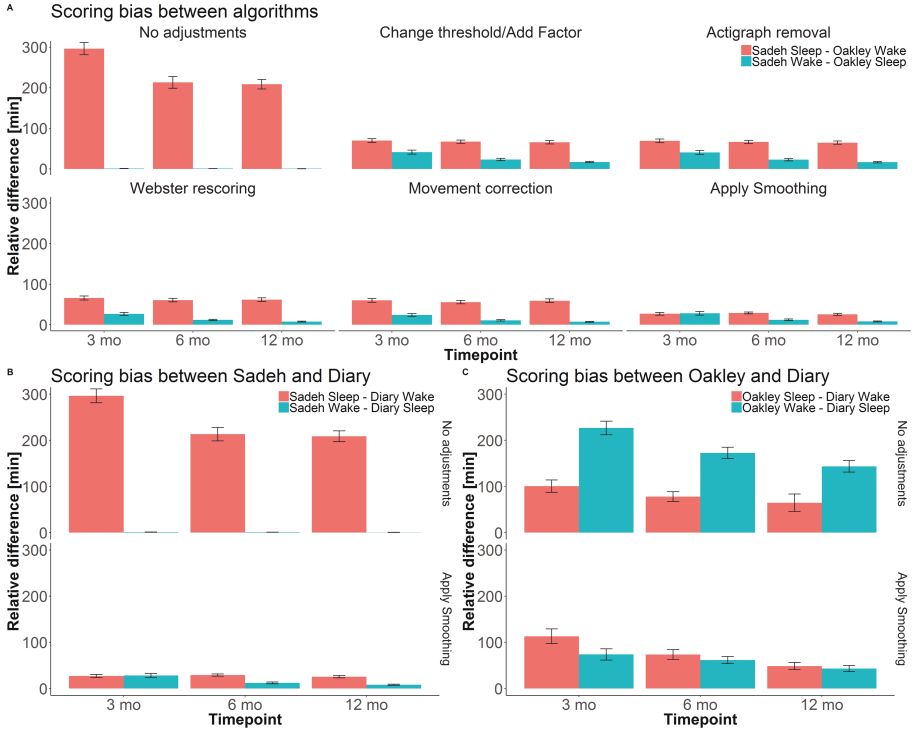

Figure 2. Sleep/wake bias of scoring algorithms and 24-hour diary reported by parents. Scoring bias shows disagreement of scoring as sleep or wake (sum of minutes within 24 hours, error bars represent 95% confidence interval). (A) Scoring bias is depicted without adjustments and for each adjustment step. (B) Scoring bias of the Sadeh algorithm and compared to the 24-hour diary is shown without adjustments and including all adjustments. (C) Bias of the Oakley algorithm compared to the diary is shown without adjustments and including all adjustments.

Table 2. Differences in sleep parameter estimates with and without adjustments estimated by Bland–Altman scores

	Sleep Duration (hour)		Day-to-Day Sleep Variability (hour)		% Night Sleep		Fragmentation (/hour)	
Age	No adjustments	After adjustments	No adjustments	After adjustments	No adjustments	After adjustments	No adjustments	After adjustments
3 months	5.45 ± 1.90	−0.01 ± 0.90	−0.09 ± 0.70	0.01 ± 0.22	−6.99 ± 5.74	0.11 ± 1.52	−0.65 ± 0.37	−0.04 ± 0.09
6 months	3.96 ± 1.69	0.33 ± 0.41	−0.06 ± 0.81	0.00 ± 0.22	−6.64 ± 6.33	−0.40 ± 1.08	−0.78 ± 0.35	−0.06 ± 0.12
12 months	4.08 ± 1.46	0.32 ± 0.37	−0.09 ± 0.55	−0.09 ± 0.20	−4.74 ± 5.60	3.35 ± 1.34	−0.92 ± 0.32	−0.17 ± 0.08

Means ± critical difference is shown. Means show general bias of one measure over the other. Critical difference show 95% differences in estimates.

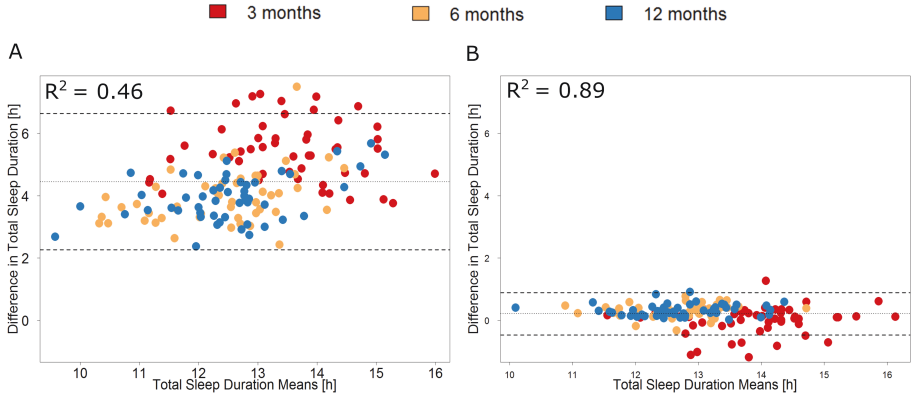

Figure 3. Bland–Altman plots of *Total Sleep Duration* estimates from Sadeh and Oakley algorithm. Each infant is represented by three dots indicating the age group by color. (A) Without adjustments, the difference in *Total Sleep Duration* is >4 hours with a critical difference of 2.18 hours, indicating that without adjustments *Total Sleep Duration* estimates from the Sadeh algorithm are 2–7 hours above estimates from the Oakley algorithm. (B) With six-step adjustments, the difference in *Total Sleep Duration* is lowered to ~ 0 hour with a critical difference of 0.68 hour.

Comparison with BISQ (Sadeh algorithm–questionnaire, Oakley algorithm–questionnaire)

We found a generally large deviation from parental questionnaire data compared to actimetry data in *Total Sleep Duration*, as indicated by a critical difference of 3.19 hours. This includes both, under- and overestimating of the objective estimates by parent's estimates (95%; see Supplementary Figures 1 and 2). For example, parents reporting their infants' sleep duration to be 13.5 hours revealed objectively measured infant sleep duration between 11.86 and 14.6 hours. However, there was no systematic bias (i.e. either under- or overestimating) *Total Sleep Duration* of their infants.

Discussion

Only standardized objective assessments reliably capture the large variability of sleep behavior in infancy, which is the most pronounced during the human lifespan [5]. When polysomnographic recording is not a feasible approach to measure sleep in real-life settings with large populations, actimetry can transform movement counts into objective sleep estimates. We applied a six-step set of adjustments to actimetry-based sleep estimation designed for infants, with the goal to overcome discrepancies in sleep estimates between existing scoring algorithms [9, 10]. The use of 24-hour diaries minimizes signal miscomputation through external factors and improves the analysis of daytime sleep. These methods will help to extend reference values based on parental reports [5] or meta-analysis based on different devices [20].

Adjustments reduced disagreement between algorithms from 16%–22% to 3%–4%. Both algorithms showed a bias when compared to the 24-hour diary and to the other algorithm: the Sadeh algorithm was biased towards sleep and the Oakley algorithm was biased towards wake. Both biases were significantly reduced by the adjustments. Such standardization is of great importance for computation of sleep variables. For example, without adjustments, *Total Sleep Duration* deviates up to 7 hours depending on the algorithm used, with higher sleep duration estimates when using the Sadeh algorithm compared to the Oakley algorithm. After adjustments, these estimates vary less than 1 hour. Importantly, this also increased the correlation, meaning that the infants who overall showed the highest sleep duration as calculated from one algorithm also are estimated to have a high sleep duration with the other algorithm. Similar effects were seen for parameters such as *% Night Sleep* and *Fragmentation*. Only *Day-to-Day Sleep Variability* showed no bias without adjustments, but even for this parameter correlation could be improved drastically.

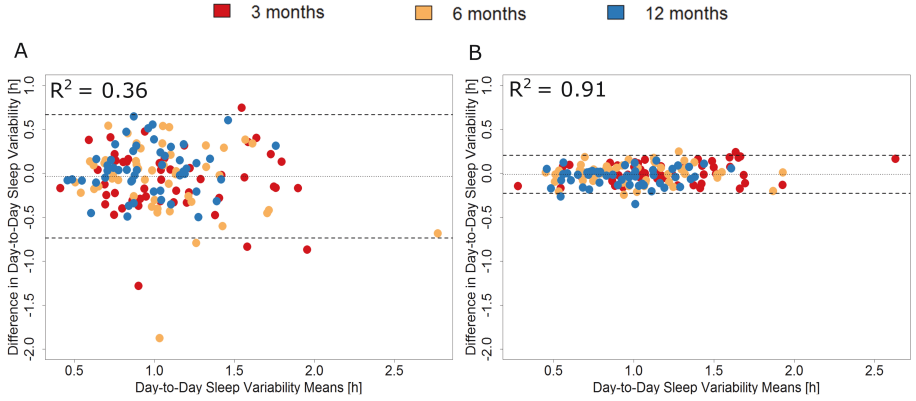

Figure 4. Bland–Altman plots showing difference in *Day-to-Day Sleep Variability* between scoring based on Sadeh and Oakley algorithms. Each infant is represented by three dots indicating the age group by color. (A) Without adjustments, the difference in *Day-to-Day Sleep Variability* is ~0 hour with a critical difference of 0.7 hour. (B) With six-step adjustments, the difference in *Day-to-Day Sleep Variability* estimate is ~ 0 hour with a critical difference of 0.22 hour.

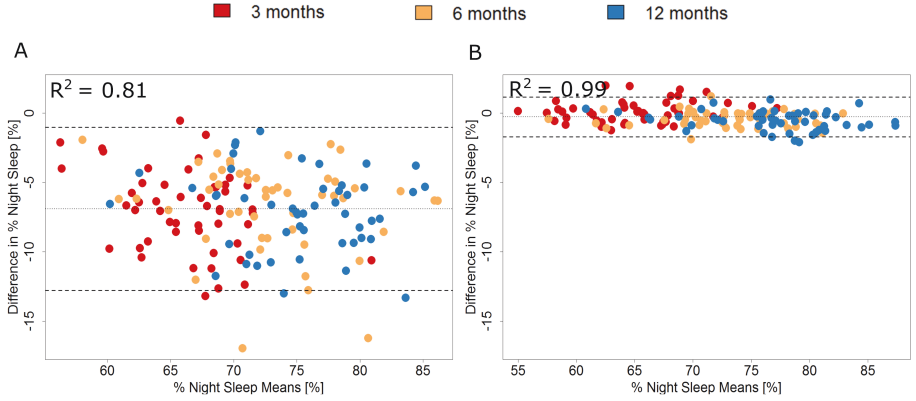

Figure 5. Bland–Altman plots showing difference in *% Night Sleep* between scoring based on Sadeh and Oakley algorithm. Each infant is represented by three dots indicating the age group by color. (A) Without adjustments, the mean difference in *% Night Sleep* estimate is ~7% with a critical difference of 5.87%. (B) With six-step adjustments, the mean difference in *% Night Sleep* estimate is ~0% with a critical difference of 1.43%.

We tested the stability across age of the approach. Effects were stable from 3 to 6 months of age in *Fragmentation*, yet no systematic effect was found in other variables. This suggests that factors compromising the algorithm output are not stable traits of the infant, e.g. small differences in activity between wake and sleep might account for differences at 3 months but does not persist through older age.

We also identified age-specific effects that affect actimetry outcomes. Scoring agreement generally increases with age. We hypothesize that this is primarily due to increased motor activity during wake as part of motor development. Another contributing factor may be the reduction of night-feedings as infants grow older. In the 24-hour diary, feeding was assigned to wake, but transitions of feeding to sleep may be blurry and this might contribute to differences between diaries and scoring. Nonetheless, benefits of algorithm adjustments still prevailed in the older infants (with fewer feedings). Additionally, external movement during sleep in very young infants can lead to mis-scoring of up to 1 hour. This was corrected by introducing our adjustments. As children get older and transit to nap only in a bed, this adjustment may become redundant. Furthermore, with increasing age, removal time of the actimeters increased (e.g. removal by child or other infants, longer periods of bathing/water activities), which led to mis-scoring of up to 30 minutes. Completing the 24-hour diary remains important for the reliable detection and correction of such incidents.

Information from the 24-hour diary support the integration of the ~20% of infant sleep that occurs during daytime. Daytime naps are often missed in traditional analyses, but they reflect

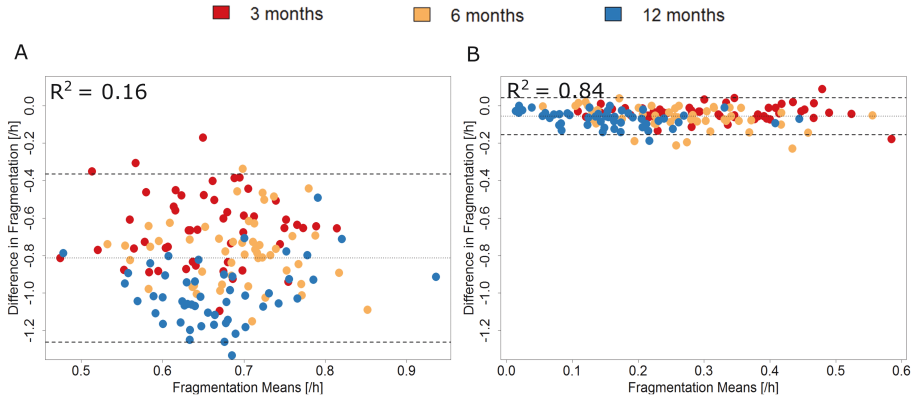

Figure 6. Bland–Altman plots showing difference in *Fragmentation* between scoring based on Sadeh and Oakley algorithm. Each infant is represented by three dots indicating the age group by color. (A) Without adjustments, the mean difference in *Fragmentation* estimate is ~0.8% with a critical difference of 0.45. (B) With six-step adjustments, the mean difference in *Fragmentation* estimate is ~0 with a critical difference of 0.1%.

Table 3. Pearson's correlations testing age effects and comparison of age groups are based on the differences between the two algorithms in sleep variables

	Sleep Duration (hour)		Day-to-Day Sleep Variability (hour)		% Night Sleep		Fragmentation (/hour)	
Correlation *r*	No adjustments	After adjustments	No adjustments	After adjustments	No adjustments	After adjustments	No adjustments	After adjustments
3–6 months	0.31	0.09	-0.21	0.009	0.31	0.001	**0.49**	0.05
3–12 months	0.09	0.13	-0.13	0.23	0.20	0.21	0.26	0.07

Bold value is significant after FDR correction.

the important build-up of sleep pressure and the neurophysiological capacity of children to increase consolidated waking bouts [21]. Our approach circumvents these difficulties by integrating complementary information from a 24-hour sleep diary. Although our semi-automated integration requires time investment of study participants and researchers, it greatly improves data reliability and allows comparison across studies. We suggest to integrate digital diaries (i.e. sleep tracking apps) linked to actimetry input for future studies. Parents should be given the opportunity to confirm sleep periods or reject faulty ones electronically. Additional computational corrections can be introduced to (1) distinguish between movements of the infant vs external movements or (2) automatically detect periods where the actimeter is not worn. This requires the integration of new sensors such as heart rate or skin temperature. Such sensors could also distinguish quiet wakefulness from sleep, which cannot be achieved with acceleration only.

Limitations

This investigation aimed at quantifying infant sleep in real-life settings and did thus not compare actimetry or 24-hour diary data with simultaneously assessed polysomnography. Polysomnography is the current gold-standard objective sleep measure, yet its recording was not feasible in the frame of the current research (50 infants, multiple recordings throughout the first year of life). A further caveat of this research is that the specific infant Sadeh algorithm is validated only against observer rating [9], in contrast to a similar algorithm, which was validated against polysomnography in young children [22, 23]. Yet, the Oakley algorithm was validated against polysomnography in infants [11]. Remaining validations are clearly needed, and we anticipate that the here proposed analytical adjustments will further increase agreement between actimetric and electrophysiological measures of sleep in infants. Principally, we investigated infant data and some of the adjustments might be specific to this age only, while others might even transfer to older age groups. A systematic investigation in older age groups would identify which adjustments support data processing and interpretation.

In conclusion, we present adjustments to standardize actimetric sleep/wake scoring for nighttime and daytime sleep. Applying these adjustments increases the reliability of measured infant sleep variables.

Supplementary material

Supplementary material is available at *SLEEP* online.

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6.3 ARTICLE 3

Which are the central aspects of infant sleep? The dynamic of sleep composites across infancy

Sarah F. Schoch, Reto Huber, Malcolm Kohler, Salome Kurth

Abstract

Sleep during infancy is important for the well-being of both infant and parent. Therefore, there is large interest in characterizing infant sleep with reliable tools, for example by combining actigraphy with 24-h-diaries. However, it is critical to select the right variables to characterize sleep. In a longitudinal investigation, we collected sleep data of 152 infants at ages 3, 6, and 12 months. Using principal component analysis, we identified five underlying sleep composites from 48 commonly-used sleep variables: *Sleep Night*,*Sleep Day*, *Sleep Activity*, *Sleep Timing*, *Sleep Variability*. These composites accurately reflect known sleep dynamics throughout infancy as *Sleep Day* (representing naps), *Sleep Activity* (representing sleep efficiency and consolidation), and *Sleep Variability* (representing day-to-day stability) decrease across infancy, while *Sleep Night* (representing nighttime sleep) slightly increases, and *Sleep Timing* becomes earlier as one ages. We uncover interesting dynamics between the sleep composites and demonstrate that infant sleep is not only highly variable between infants but also dynamic within infants across time. Interestingly, *Sleep Day* is associated with behavioral development and therefore a potential marker for maturation. We recommend either the use of sleep composites or the core representative variables within each sleep composite for more reliable research.

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sensors

MDPI

Article

Which Are the Central Aspects of Infant Sleep? The Dynamics of Sleep Composites across Infancy

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Abstract: Sleep during infancy is important for the well-being of both infant and parent. Therefore, there is large interest in characterizing infant sleep with reliable tools, for example by combining actigraphy with 24-h-diaries. However, it is critical to select the right variables to characterize sleep. In a longitudinal investigation, we collected sleep data of 152 infants at ages 3, 6, and 12 months. Using principal component analysis, we identified five underlying sleep composites from 48 commonly-used sleep variables: *Sleep Night*, *Sleep Day*, *Sleep Activity*, *Sleep Timing*, *and Sleep Variability*. These composites accurately reflect known sleep dynamics throughout infancy as *Sleep Day* (representing naps), *Sleep Activity* (representing sleep efficiency and consolidation), and *Sleep Variability* (representing day-to-day stability) decrease across infancy, while *Sleep Night* (representing nighttime sleep) slightly increases, and *Sleep Timing* becomes earlier as one ages. We uncover interesting dynamics between the sleep composites and demonstrate that infant sleep is not only highly variable between infants but also dynamic within infants across time. Interestingly, *Sleep Day* is associated with behavioral development and therefore a potential marker for maturation. We recommend either the use of sleep composites or the core representative variables within each sleep composite for more reliable research.

Keywords: actimetry; sleep assessment; maturation; sleep variables; variable selection

1. Introduction

Why is sleeping the most common behavior of an infant in its first year of life [1]? Sleep fulfills an important function in development. The neurophysiology of sleep is linked to brain maturation, neural reorganization [2–4], as well as processes of learning and memory [5,6] (for an overview see [7]). However, aside from the vital importance of sleep for a child, it also affects the quality of infant-parent bonds, as early periods with infant sleep problems have been linked to parental depression and stress [8,9]. Supporting healthy infant sleep can thus improve the wellbeing of the whole family.

Sleep-wake patterns are extensively diversified across infants—and vary to a much greater extent compared to any other period in life [1]. This inter-individual variability makes the establishment of normative age-specific sleep values more difficult. Additionally, sleep is not a one-dimensional construct, but characterized by numerous dimensions of quantity, quality, timing, or consolidation. While sleep undergoes drastic changes across infancy, not all sleep dimensions evolve at the same time

or to the same degree. Possibly the most recognizable is the alteration from sleep being distributed throughout the 24-h-day (polyphasic sleep) to one primary sleep phase at nighttime (monophasic sleep)—a transition happening gradually from birth until about 5 years of age [1]. This transition involves a multitude of changes, as it affects not only the timing of sleep, but also its depth [10] and fragmentation [11]. Additionally, sleep quantity, measured as total sleep duration across 24 h, also decreases by ~8 min per month across the first year of life [12]. Notably, alongside the changes in sleep behavior, the neurophysiology of sleep is reorganized and the composition of sleep states change across the first years of life: Rapid eye movement (REM) sleep becomes less predominant and the electrophysiological characteristics typical for adult sleep (sleep spindles, slow waves) emerge [13,14].

Due to the ubiquity of sleep and its importance in early development, it is unsurprising that there is large scientific interest in infant sleep. Researchers use both subjective methods (questionnaires e.g., the Brief Infant Sleep Questionnaire ([BISQ) and 24-h-sleep-wake-diaries [15]) and objective methods (actigraphy [16], videosomnography [17], and polysomnography [18]). Each method has advantages and disadvantages [19]. There is only moderate agreement among the diverse methods, with larger discrepancies between questionnaires vs. objective data than between 24-h-diaries vs. objective data [20,21]. Subjective methods are cost effective and easy to administer to large populations. Yet, they are limited to items parents are aware of (e.g., sleep behavior but not sleep stages) and might be biased by parent perception. Furthermore, the selection of assessment method largely depends on the research question and available resources. Objective methods reduce subjective bias and represent the different dimensions of sleep. Over the past 25 years, the combination of actigraphy with 24-h-diaries has emerged as the preferred method for many infant sleep investigations [22]. Its advantage is the combination of objective and subjective data that allows for the quantification of sleep in large populations and in natural environments, while being cost-effective [22,23]. However, issues remain regarding the standardization of actigraphy, especially in infants and young children [22,24]. However, a remaining issue lies in capturing the dimensions of sleep accurately.

One current issue in investigating infant sleep is the selection of sleep variables. On one hand, there are several possible sleep domains and thus numerous sleep variables that can be calculated. On the other hand, the computation of these sleep variables is not standardized. The current situation leaves researchers to decide which sleep variable and computations to choose [22]. For example, sleep duration is one of the most investigated sleep behaviors in infancy (reported in 82% of studies [12]). However, reports are based on different concepts, such as sleep duration computed across night time only, sleep duration including 24 h, duration of sleep with a split of day/night at a chosen clock time, or with clock times for day/night split that are individually assigned for each infant. This divergence is problematic because it prevents comparability across studies [25]. It is also a likely source for lacking reproducibility. Additionally, researchers might rely on default variables from an automated analysis program, which is dubious if the research question demands more specificity. Using a large number of sleep variables to address the dimensions of sleep will likely increase false positives due to multiple testing [26]. Therefore, one should aim for a reduction of methodological complexity.

A novel and promising approach to handle the complexity of sleep dimensions was recently presented. Based on the data of young children, Staples et al. proposed “sleep composites” that were combined from multiple commonly-used sleep variables. This approach reduces the dependence on single (often overlapping) sleep variables and increases the measurement stability [27]. A total of four sleep composites were discovered in both children and their mothers. These sleep composites contain the key dimensions of sleep: *Sleep Duration*, reflecting the quantity of sleep during the night; *Sleep Timing*, reflecting bedtimes and sleep onset times; *Sleep Variability*, reflecting day-to-day differences in sleep timing and duration; and *Sleep Activity*, reflecting movements and awakenings during the night. They also found that daytime sleep and sleep latency were separate constructs to these four sleep composites (i.e., loaded on their own composite). The identified sleep composites revealed higher consistency across different assessment timepoints compared to single sleep variables. A higher consistency is important to anchor sleep behaviors as reference in certain age periods, which is

crucial, for example, to unravel the influence of early sleep variables on later regulatory, cognitive, or emotional outcomes.

- The goal of this study was to first extend the approach of Staples et al. to an infant dataset and to, secondly, facilitate variable selection for future sleep studies;
- We therefore included 48 single sleep variables, which thoroughly characterize the diverse dimensions of sleep, and then performed a component analysis to identify the core infant sleep composites;
- We then examined the evolution of the sleep composites across repeated assessments throughout the first year of life and also tested for sex differences in the sleep composites. Additionally, we explored the stability of composites as well as the stability of the single sleep variables;
- Finally, to evaluate the relevance of sleep for development and to identify maturational markers, we linked sleep composites to infant behavioral developmental scores.

2. Materials and Methods

2.1. Participants

A total of 152 healthy infants (69 female) in Switzerland participated in a longitudinal study on infant sleep and behavioral development. Of these, a subsample of 50 infants were included in a previous investigation [24]. Caregivers and participants were recruited through maternity wards, midwifes, pediatricians, daycares, letters, social media, personal contacts, and flyers distributed at universities, libraries, supermarkets, schools, family organizations, and community centers. Participants were screened for study eligibility by means of an online questionnaire or telephone interview. Inclusion criteria for infants were good general health, being primarily breastfed at time of inclusion (i.e., inclusion criterium of at least 50% of daily nutrition intake through breastfeeding at the first assessment at the age of 3 months), vaginal birth (no cesarean section), and birth within 37–43 weeks of gestation. Parents were required to have a good knowledge of the German language.

Exclusion criteria for infants were disorders of the central nervous system, acute pediatric disorders, brain damage, chronic diseases, as well as family background of narcolepsy, psychosis, or bipolar disorder. Infants with birth weight below 2500 g, intake of medication affecting the sleep-wake cycle, or antibiotics prior to the first assessment were also excluded.

Ethical approval was obtained from the cantonal ethics committee (BASEC 2016-00730) and study procedures were consistent with the declaration of Helsinki. Written parental consent was obtained after an explanation of the study protocol and before enrollment.

2.2. Experimental Design

We assessed 152 infants longitudinally at the ages 3, 6, and 12 months. We scheduled assessments within a 1-month window around the target age, therefore actual age at the start of assessment was between 2.43–3.39 months, 5.42–6.28 months, and 11.47–12.26 months.

We comprehensively quantified sleep-wake behavior for 11 continuous days. It has previously been suggested that 7 days of recording duration are required to then obtain 5 “complete” and artifact-free days to be included in analysis in children 1–5 years of age [28]. We expected further increased data loss in infants and therefore extended the recording length in our study to 11 days. Ankle actigraphy and a 24-h-diary were simultaneously acquired during each of the three assessments, in alignment with our published recommendations for studying this age group [23]. GENEActiv movement sensors “actigraphs” (Activinsights Ltd., Kimbolton, UK, 43 × 40 × 13 mm, MEMS sensor, 16 g, 30 Hz Frequency recording resolution), which are sensitive to +/− 8 g range at 3.9 mg resolution, were attached to the infant’s left ankle in a modified sock (pocket sewn onto its side) or with a Tyvek paper strap. Parents were instructed to only remove the actigraph for bathing/swimming activities and to document any removal of the actigraph in the 24-h-diary. In the 24-h-diary (adapted from [21]), parents reported in 15-min intervals on infant sleep and external movement occurring during infant

sleep, e.g., sleeping in the parents arms, stroller, or baby sling etc. Further recorded parameters included feeding, crying episodes (>15 min), and bedtimes (putting infant to bed in the evening and picking it up from the bed in the morning).

Additionally, in online questionnaires, parents reported information on family background, health, and demographics. Families received small gifts for their participation.

2.3. Behavioral Development

Behavioral developmental status was assessed with the age-appropriate Ages and Stages questionnaire (ASQ) [29]. A *Collective Score*, represented by the sum of scores across five sub-domains (*Communication*, *Gross Motor*, *Fine Motor*, *Problem Solving*, and *Personal Social*), was computed to quantify overall development. Additionally, we analyzed *Personal Social* and *Gross Motor* individually because these subscales correlated with the well-validated testing battery Bayley Scales of Infant Development [30] and specifically also because these two sub-domains can indicate developmental delay [29,31]. Participants whose questionnaire was completed later than 1 week after the last day of the corresponding assessment were excluded from analysis and missing data was inputted (Section 2.4.2).

2.4. Sleep Analysis

2.4.1. Sleep–Wake-Behavior

Actigraphy data was processed according to our standard protocols [24]. Binary data were extracted using GENEactiv PC Software (Version 3.1), imported into Matlab (R2016b), and converted to activity counts [32]. The conversion included a 3–11 Hz bandpass filter and signal compression to 15 s bins. Acceleration data from the three movement axes was combined using sum of squares. The signal was then compiled to one data point per minute (analysis resolution). A published algorithm [33] was used to identify infant sleep and wake periods, and a 6-step modification [24] was applied to refine prediction for a better fit with the 24-h-diary. The first step of the modification (distinction between periods of high and low activity) was changed to use a threshold of 'mean activity * 0.72'. Time periods without actigraphy information (i.e., when the actigraph was not worn) were identified through the 24-h-diary or visual inspection (abrupt periods of no activity). These were, whenever possible, replaced with information on sleep or wake from the 24-h-diary.

2.4.2. Handling of Missing Data

For some infants no sleep data was available for all timepoints: $n = 2$ at 3 months (study enrollment at later age), $n = 4$ at 6 months ($n = 3$ device failure, $n = 1$ parent withdrew from sleep assessment part of study), and $n = 9$ at 12 months ($n = 2$ device failure, $n = 3$ participant attrition, $n = 2$ parent withdrew from sleep assessment part of study, $n = 1$ family moved away, $n = 1$ chronic sickness). Participants were instructed to collect actigraphy data for the duration of 11 continuous days (i.e., putting actigraph on before bedtime on the first day and removing it after getting up on the last day). Yet sickness and vacation of participants as well as device failure prevented the full 11-day recording in some cases ($n = 10$ at 3 months, $n = 28$ at 6 months, $n = 28$ at 12 months). Furthermore, in some instances the recording period was extended beyond the 11 days (e.g., because the original device was temporarily lost or parents recorded longer, $n = 27$ at 3 months, $n = 23$ at 6 months, $n = 15$ at 12 months). Therefore, recordings with available data for both the actigraphy and 24-h-diary lasted on average 10.76 ± 1.72 days: 11.13 ± 1.17 days at 3 months, 10.60 ± 1.91 at 6 months, and 10.55 ± 1.93 at 12 months. Additionally, single days were excluded if infants were either sick (except for common cold symptoms) or if the actigraph was removed for a longer time duration (1 h for partial-day variables, 3 h for entire-day variables, 5 min for variables relying on movement counts, and for clock time variables (e.g., Sleep Onset) a 30-min time window centered on the clock time of parent-reported infant sleep on- or offset, see Supplementary Table S1). Single days were furthermore excluded if the fit between actigraphy-based data and 24-h-diary was poor (see Supplementary Table S1).

2.4.3. Calculation of Sleep Variables

To capture the multitude of dimensions of infant sleep, we calculated 48 sleep variables of interest, based on previous definitions [22,27,34] (Table 1). A total of 3 valid recording days of actimetry were set as the minimum to compute sleep variables in each participant. We selected 3 valid recording days because acceptable reliability for most sleep variables (>0.70) was reported regarding a majority of sleep variables at an infant age of 12 months [28]. For variability variables, a minimum of 5 valid recording days was required, which was chosen in order to maximize the included data and reliable estimates. All calculated sleep variables (except variability variables which were standard deviations across days) were averaged across all valid recorded days. After calculating sleep variables, additional exclusions were performed: For time zone change of >1 h less than 1 week before the recording (n = 1 at 12 months), for medication affecting sleep (n = 2 at 3 months), and for medical problems (n = 1 at 6 months, n = 2 at 12 months) or psychological trauma experienced (n = 1 at 12 months).

Table 1. Definition and descriptive statistics at 3, 6, and 12 months of the 48 infant sleep variables based on the 24-h-diary and actigraphy that entered the principal component analysis. *Bedtime* and *Get up Time* and their variability variables are based on parent report in their 24-h-diary, all other variables are based on actigraphy (with adjustments from diaries as reported in Section 2.4.1). Clock-time variables are reported in two formats: clock times and minutes per day. Mean ± Standard Deviation (Minimum – Maximum).

Variable Name	3 Months	6 Months	12 Months
(1) *Bedtime* (clock time in min) Parent-reported time in the 24-h-diary of putting the child to bed. For missing values, the first minute of reported sleep was used. If bedtime exceeded midnight 1440 was added.	21:14 ± 01:15 (18:56–01:19) 1273.71 ± 75.38 (1116–1479)	20:27 ± 01:19 (18:25–00:21) 1226.59 ± 69.18 (1105–1420.8)	20:21 ± 00:53 (18:40–00:16) 1220.78 ± 53.46 (1120–1416)
(2) *Variability of Bedtime* (SD) Standard deviation of *Bedtime* across recording days.	00:44 ± 00:22 (0:00–01:58) 43.5 ± 21.64 (0–118.39)	00:33 ± 00:18 (0:00–01:27) 32.6 ± 17.76 (0–87.13)	00:29 ± 00:17 (0:03–01:31) 28.88 ± 16.83 (3.35–91.41)
(3) *Get up Time* (clock time in min) Parent reported time in the 24-h-diary of getting out of bed in the morning. For missing values, the last minute of reported sleep was used.	07:53 ± 00:52 (06:02–10:12) 472.88 ± 52.06 (361.5–612)	07:24 ± 00:49 (05:36–09:38) 443.84 ± 48.89 (335.5–577.7)	07:18 ± 00:43 (05:23–10:25) 437.93 ± 43.41 (323.33–625)
(4) *Variability of Get up Time* (SD) Standard deviation of *Get up Time* across recording days.	00:42 ± 00:17 (00:06–01:36) 42.02 ± 16.44 (6.35–96.17)	00:36 ± 00:16 (0:00–01:49) 36.35 ± 16.26 (0–109.2)	00:36 ± 00:17 (00:06–01:40) 35.8 ± 16.93 (6.12–99.82)
(5) *Sleep Onset* (clock time in min) Following *Bedtime*, the first minute asleep of at least 10 min of consecutive sleep. If asleep at *Bedtime*, the first minute asleep before *Bedtime* was chosen.	20:58 ± 01:08 (18:47–00:33) 1257.94 ± 68.08 (1127.17–1473.3)	20:29 ± 01:06 (18:47–00:09) 1228.59 ± 65.87 (1112.33–1449.4)	18:49 ± 00:55 (18:46–23:24) 1228.74 ± 54.7 (1125.5–1423.6)
(6) *Variability of Sleep Onset* (SD) Standard deviation of *Sleep Onset* across recording days.	00:52 ± 00:24 (00:08–02:37) 51.87 ± 23.83 (7.75–157.33)	00:38 ± 00:19 (00:06–01:49) 37.79 ± 19.07 (6.33–109.24)	00:34 ± 00:17 (00:05–01:20) 34.28 ± 17.2 (4.7–79.86)
(7) *Sleep Latency* (min) Duration in minutes between *Bedtime* and *Sleep Onset*, set to 0 if *Sleep Onset* is before *Bedtime*.	7.79 ± 7.97 (0–42)	11.29 ± 8.3 (0–45.5)	10.94 ± 8.2 (0–38.38)
(8) *Variability of Sleep Latency* (SD) Standard deviation of *Sleep Latency* across recording days.	10.29 ± 9.17 (0–56.64)	11.43 ± 7.61 (0–44.91)	10.26 ± 7.14 (0–40.27)

Table 1. *Cont.*

Variable Name	3 Months	6 Months	12 Months
(9) *Sleep Offset* (clock time in min) Last minute asleep of at least 10 consecutive minutes asleep before *Get up Time* or if asleep at *Get up Time* last minute asleep after *Get up Time*.	07:51 ± 00:53 (05:55–10:23) 470.93 ± 53.13 (354.8–622.86)	07:18 ± 00:49 (05:25–09:43) 437.71 ± 49.41 (324.5–583.38)	07:17 ± 00:47 (05:16–10:16) 437.96 ± 46.6 (316.67–615.5)
(10) *Variability of Sleep Offset* (SD) Standard deviation of *Sleep Offset* across recording days.	00:48 ± 00:19 (00:16–01:54) 48.06 ± 18.51 (15.82–114.17)	00:40 ± 00:17 (00:17–02:03) 39.74 ± 16.89 (16.51–123.17)	00:39 ± 00:21 (00:12–02:45) 38.66 ± 20.5 (12.02–164.83)
(11) *Midsleep* (clock time in min) Midpoint between *Sleep Onset* and *Sleep Offset*.	02:24 ± 00:53 (00:33–04:59) 143.93 ± 53.02 (32.65–298.7)	01:53 ± 00:52 (00:14–04:53) 112.88 ± 51.71 (13.7–293.81)	01:54 ± 00:46 (00:14–04:38) 114.03 ± 46.09 (13.5–278.2)
(12) *Variability of Midsleep* (SD) Standard Deviation of *Midsleep* across recording days.	00:38 ± 00:14 (00:12–01:19) 37.62 ± 13.62 (11.95–78.54)	00:28 ± 00:11 (00:09–01:04) 28.37 ± 11.24 (8.86–64.22)	00:27 ± 00:13 (00:08–01:42) 27.33 ± 13.46 (7.7–101.79)
(13) *Sleep Opportunity* (min) Time between *Bedtime* and *Get Up Time* (unless asleep at either of these times, in which case *Sleep Onset*/*Sleep Offset* was used).	662.46 ± 62.8 (494.2–840)	670.89 ± 54.38 (558.11–820.67)	666.4 ± 42.51 (578–738)
(14) *Variability of Sleep Opportunity* (SD) Standard deviation of *Sleep Opportunity* across recording days.	62.85 ± 23.14 (16.83–124.55)	47.29 ± 20.18 (12.31–129.93)	45.32 ± 23.34 (14.51–163.62)
(15) *Sleep Period* (min) Time between *Sleep Onset* and *Sleep Offset*.	651.87 ± 58.05 (488.9–796)	651.52 ± 50.44 (543.56–821.78)	650.94 ± 44.7 (547–735.75)
(16) *Variability of Sleep Period* (SD) Standard deviation of *Sleep Period* across recording days.	67.09 ± 22.21 (21.19–127.12)	51.83 ± 21.73 (16.36–144.75)	49.85 ± 23.43 (11.27–163.32)
(17) *Total Sleep Time* (min) Minutes scored 'Sleep' within *Sleep Period*.	573.63 ± 58.25 (421.33–709.44)	605.23 ± 47.38 (492.44–728.75)	627.3 ± 51.29 (488.5–717.1)
(18) *Variability of Total Sleep Time* (SD) Standard deviation of *Total Sleep Time* across recording days.	53.47 ± 18.91 (18.62–108.3)	45.98 ± 16.04 (10.15–91.2)	46.33 ± 19.44 (11.61–140.08)
(19) *Sleep Efficiency* (%) (*Total Sleep Time*)/(*Sleep Opportunity*) × 100.	87.83 ± 5.4 (69.37–99.5)	90.67 ± 4.08 (80.55–99.21)	94.25 ± 3.52 (84.06–99.64)
(20) *Variability of Sleep Efficiency* (SD) Standard deviation of *Sleep Efficiency* across recording days.	5.46 ± 2.35 (1.84–18.22)	4.59 ± 1.95 (1.57–11.97)	3.57 ± 1.9 (0.45–12.94)
(21) *Wake after Sleep Onset* (min) Minutes scored 'Wake' in *Sleep Period*.	69.04 ± 32.15 (13.7–197.75)	44.73 ± 24.55 (1.86–121.17)	22.31 ± 17.02 (0–78.22)
(22) *Variability of Wake after Sleep Onset* (SD) Standard deviation of *Wake after Sleep Onset* across recording days.	32.72 ± 12.52 (11.3–79.02)	26.63 ± 13.46 (4.26–86.03)	18.8 ± 10.68 (1.9–57.17)
(23) *Longest Nocturnal Wake* (min) Longest period scored 'Wake' followed by at least 15 min scored 'Sleep' in *Sleep Period*.	31.87 ± 14.5 (7.6–93.33)	24.37 ± 13.19 (1.86–75.89)	13.74 ± 9.65 (0–56.67)
(24) *Variability of Longest Nocturnal Wake* (SD) Standard deviation of *Longest Nocturnal Wake* across recording days.	16.43 ± 7.32 (3.1–39.7)	17.09 ± 10.2 (3.14–48.35)	12.04 ± 8.78 (0–60.19)
(25) *Nocturnal Wake Frequency per Hour* (waking/hour) (Number of Nocturnal Wake Periods in *Sleep Period*)/*Sleep Period*.	0.34 ± 0.11 (0.1–0.61)	0.23 ± 0.1 (0.02–0.49)	0.14 ± 0.09 (0–0.46)
(26) *Variability of Nocturnal Wake Frequency per Hour* (SD) Standard deviation of *Nocturnal Wake Frequency per Hour* across recording days.	0.12 ± 0.04 (0.03–0.27)	0.11 ± 0.04 (0.03–0.22)	0.1 ± 0.04 (0–0.24)

Table 1. *Cont.*

Variable Name	3 Months	6 Months	12 Months
(27) *Variability of Activity level* (SD) Standard deviation of activity per minute in *Sleep Period*.	168.64 ± 61.51 (40.69–356.73)	178.91 ± 79.21 (49.92–478.14)	108.5 ± 50.86 (33.64–336.46)
(28) *Percent Active Epochs* (ratio) (Minutes of epochs with non-zero activity in *Sleep Period*)/*Sleep Period*.	0.3 ± 0.05 (0.14–0.4)	0.24 ± 0.04 (0.13–0.34)	0.23 ± 0.03 (0.14–0.32)
(29) *Variability Percent Active Epochs* (SD) Standard deviation of *Percent Active Epochs* across recording days.	0.04 ± 0.01 (0.02–0.09)	0.04 ± 0.02 (0.01–0.1)	0.04 ± 0.01 (0.01–0.12)
(30) *Longest Sleep* (min) Longest continuous period scored as 'Sleep'.	292.19 ± 90.16 (139–580.29)	339.21 ± 99.18 (158.75–632.17)	458.24 ± 126.82 (166.89–706.44)
(31) *Variability of Longest Sleep* (SD) Standard deviation of *Longest Sleep* across recording days.	82.75 ± 36.25 (21.04–190.74)	104.82 ± 39.39 (23.02–222.59)	127.41 ± 50.7 (14.21–245.62)
(32) *Longest Wake* (min) Longest continuous period scored as 'Wake'.	162.44 ± 27.74 (101.11–292.29)	212.13 ± 32.94 (139.33–348)	293.14 ± 40.25 (195.33–402)
(33) *Variability of Longest Wake* (SD) Standard deviation of *Longest Wake* across recording days.	40.07 ± 18 (11.65–111.57)	50.11 ± 22.36 (9.07–109.98)	65.91 ± 22.99 (18.63–150.2)
(34) *Nap Counter* Number of daytime sleep periods exceeding 20 min between *Sleep Offset* and *Sleep Onset*.	4.06 ± 0.77 (2–6.25)	3.2 ± 0.59 (1.38–4.56)	2.07 ± 0.55 (0.67–3.57)
(35) *Variability Nap counter* (SD) Standard deviation of *Nap counter* across recording days.	1.1 ± 0.31 (0.38–2.32)	0.84 ± 0.3 (0–1.72)	0.74 ± 0.27 (0–1.9)
(36) *Sleep after Wake Onset* (min) Minutes scored Sleep between *Sleep Offset* and *Sleep Onset*.	247.56 ± 53.3 (123–382.88)	179.03 ± 37.26 (95.6–298.43)	142.54 ± 38.17 (70.5–282.63)
(37) *Variability Sleep after Wake Onset* (SD) Standard deviation of *Sleep after Wake Onset* across recording days.	61.92 ± 20.6 (19.22–154.89)	45.27 ± 16.89 (15.49–112.18)	43.76 ± 17.8 (10.94–140.06)
(38) *Sleep Duration 24 h* (min) Minutes scored 'Sleep' across 24 h.	822.19 ± 55.68 (672.86–975.44)	783.15 ± 44.45 (654–922.33)	767.64 ± 45.34 (609.67–867.43)
(39) *Variability of Sleep Duration 24 h* (SD) Standard deviation of *Sleep Duration 24 h* across recording days.	66.34 ± 19.58 (28.03–123.61)	58.77 ± 19.91 (24.73–121.56)	54.17 ± 18.89 (20.77–127.09)
(40) *Sleep Duration Day* (min) Minutes scored 'Sleep' between 7 am to 7 pm.	278.59 ± 44.98 (158.7–396)	203.46 ± 39.66 (115.88–345.13)	165.83 ± 41.31 (81.33–298.56)
(41) *Variability Sleep Duration Day* (SD) Standard deviation of *Sleep Duration Day* across recording days.	53.42 ± 16.05 (17.33–125.21)	43.44 ± 14.42 (15.79–83.25)	43.2 ± 12.34 (21.21–76.36)
(42) *Sleep Duration Night* (min) Minutes scored 'Sleep' between 7 pm to 7 am.	548.43 ± 45.54 (407.22–644.44)	579.11 ± 49.17 (426.75–672.11)	602.1 ± 47.78 (467.89–699.11)
(43) *Variability of Sleep Duration Night* (SD) Standard deviation of *Sleep Duration Night* across recording days.	44.78 ± 16.37 (12.78–92.5)	40.95 ± 14.53 (12.34–103.61)	35.51 ± 13.72 (7.31–72.05)
(44) *% Sleep Duration Night* (ratio) *(Sleep Duration Night)/(Sleep Duration 24 h)*.	0.67 ± 0.05 (0.55–0.84)	0.74 ± 0.05 (0.57–0.85)	0.78 ± 0.05 (0.61–0.88)
(45) *Variability % Sleep Duration Night* (SD) Standard deviation of *% Sleep Duration Night* across recording days.	0.05 ± 0.01 (0.02–0.09)	0.05 ± 0.01 (0.02–0.11)	0.05 ± 0.01 (0.02–0.1)

Table 1. *Cont.*

Variable Name	3 Months	6 Months	12 Months
(46) *Sleep Regularity Index Whole Day* (ratio) The probability of being in the same state (Sleep or Wake) computed for each minute, averaged across one day, and then across all recording days. Represented with ratio (0–1) ('Sleep'/'Wake), where 1 reflects the exact same rhythm every day.	0.77 ± 0.03 (0.66–0.84)	0.82 ± 0.03 (0.74–0.89)	0.87 ± 0.03 (0.76–0.95)
(47) *Sleep Regularity Index Day* (ratio) *Sleep Regularity Index* for the clock times from 7 am to 7 pm.	0.7 ± 0.04 (0.62–0.86)	0.76 ± 0.04 (0.64–0.91)	0.81 ± 0.04 (0.7–0.92)
(48) *Sleep Regularity Index Night* (ratio) *Sleep Regularity Index* for the clock times from 7 pm to 7 am.	0.84 ± 0.05 (0.66–0.96)	0.88 ± 0.05 (0.62–0.97)	0.92 ± 0.04 (0.79–0.99)

2.4.4. Data Imputation

Subsequent analyses were done in R (version 3.5.0) [35] and RStudio (version 1.1.463) [36], with several packages for data handling (tidyr, eeptools, reshape, dplyr, lubridate, phyloseq, VIM, margrittr, chron, kableExtra, knitr, and qwraps2) and plotting (corrplot, ggplot2, lattice, ggfortify, sjPlot, and cowplot) [37–54]. Missing and excluded data were inputted using multiple imputation in the mice package [55] and additional functions from miceadds, MKmisc, and micemd package [56–58]. Missing data ranged from 0% to 22.32% per variable. The dataset used for imputation included all sleep variables and several demographic variables (see Appendix A). All numerical variables were predicted using the method "2l.pmm", using the participant ID as the grouping variable and assessment age (3/6/12 months) as slope. Binary variables were predicted using the method "logreg" and categorical variables were predicted using either "polyreg" or "polyr". Two-level structure was not included in binary and categorical variable prediction. A total of 100 imputations were run with 100 iterations each using 5 cores (20 imputations per core). Data quality of the imputations were visually controlled with density plots (observed vs. imputed values) and line plots for a convergence of iterations. The reported method and prediction matrix were chosen due to best fit of the density plot.

2.4.5. Sleep Composites

We used an integrative and data-driven approach to congregate the 48 infant sleep variables (such as *Total Sleep Time*, see Table 1 for full description) to the core composite scores, inspired by an approach in young children [27]. We applied principal component analysis (PCA) with promax rotation (psych package [59]) across all participants and all assessment timepoints. Since we included more variables than Staples et al., we examined the best solution with scree and parallel plots as well as the interpretability of the resulting composites, which suggested a 5-component solution. We removed single sleep variables with absolute factor loadings below 0.512 as recommended for sample sizes exceeding 100 [60]. This led to the exclusion of 14 variables (see Table 2). Additionally, we excluded *Sleep Duration 24 h* (min, minutes scored 'Sleep' across 24 h) for interpretability (details below). In total, 33 variables were included in the final PCA solution, with 3 to 10 single sleep variables assigned to each sleep composite (Table 2).

Table 2. Single sleep variables and PCA solution with oblique rotation (*promax* rotation). The numbers in parentheses link the single sleep variables to their explanation in Table 1. Values in bold indicate the strongest loading. A total of 14 variables were excluded due to their low loading (<0.512) on any of the sleep composites. These were (7) *Sleep Latency* (min), (8) *Variability of Sleep Latency* (SD), (20) *Variability of Sleep Efficiency*, (26) *Variability of Nocturnal Wake Frequency per Hour* (SD), (29) *Variability Percent Active Epochs* (SD), (31) *Variability of Longest Sleep* (SD), (35) *Variability Nap counter* (SD), (37) *Variability Sleep after Wake Onset* (SD), (39) *Variability of Sleep Duration 24 h* (SD), (41) *Variability Sleep Duration Day* (SD), (42) *Sleep Duration Night* (min), (43) *Variability of Sleep Duration Night* (SD), (45) *Variability % Sleep Duration Night* (SD), and (46) *Sleep Regularity Index Whole Day* (Ratio).

Variables	Sleep Activity	Sleep Variability	Sleep Day	Sleep Timing	Sleep Night
(19) *Sleep Efficiency* (%)	**−0.89**	0.05	0.00	−0.01	−0.02
(23) *Longest Nocturnal Wake* (min)	**0.88**	0.04	0.05	0.02	0.19
(21) *Wake after Sleep Onset* (min)	**0.86**	−0.01	−0.11	0.00	0.16
(25) *Nocturnal Wake Frequency per Hour* (wakings/hour)	**0.79**	−0.13	−0.14	0.03	−0.13
(30) *Longest Sleep* (min)	**−0.77**	0.09	0.04	−0.03	0.15
(27) *Variability of Activity level* (SD)	**0.76**	−0.05	0.10	0.01	0.02
(22) *Variability of Wake after Sleep Onset* (SD)	**0.72**	0.09	0.10	0.01	0.05
(24) *Variability of Longest Nocturnal Wake* (SD)	**0.70**	0.07	0.28	−0.05	0.02
(48) *Sleep Regularity Index Night* (ratio)	**−0.69**	−0.18	0.17	0.04	0.09
(28) *Percent Active Epochs* (ratio)	**0.58**	−0.08	−0.28	0.01	0.12
(14) *Variability of Sleep Opportunity* (SD)	−0.05	**0.87**	−0.07	−0.08	0.04
(16) *Variability of Sleep Period* (SD)	0.04	**0.86**	0.03	−0.11	−0.07
(18) *Variability of Total Sleep Time* (SD)	−0.06	**0.73**	0.10	0.00	0.00
(10) *Variability of Sleep Offset* (SD)	−0.09	**0.73**	−0.05	0.02	0.11
(12) *Variability of Midsleep* (SD)	0.00	**0.73**	−0.08	−0.01	−0.05
(4) *Variability of Get up Time* (SD)	−0.13	**0.72**	0.02	0.14	0.19
(6) *Variability of Sleep Onset* (SD)	0.10	**0.64**	−0.04	0.06	−0.10
(2) *Variability of Bedtime* (SD)	0.20	**0.58**	0.05	−0.02	−0.11
(32) *Longest Wake* (min)	−0.10	0.04	**−0.92**	0.11	−0.17
(34) *Nap Counter*	0.03	−0.06	**0.86**	−0.12	−0.14
(36) *Sleep after Wake Onset* (min)	0.00	0.01	**0.82**	−0.11	−0.26
(47) *Sleep Regularity Index Day* (ratio)	−0.02	−0.24	**−0.76**	0.07	−0.02
(40) *Sleep Duration Day* (min)	−0.01	0.11	**0.72**	0.32	0.05
(33) *Variability of Longest Wake* (SD)	0.06	0.12	**−0.68**	0.03	−0.21
(44) *% Sleep Duration Night* (ratio)	−0.12	−0.05	**−0.56**	−0.39	0.12
(9) *Sleep Offset* (clock time in min)	0.03	−0.01	0.03	**1.01**	0.32
(3) *Get up Time* (clock time in min)	0.08	−0.03	0.01	**0.97**	0.37
(11) *Midsleep* (clock time in min)	−0.02	−0.01	0.08	**0.93**	−0.18
(5) *Sleep Onset* (clock time in min)	−0.05	−0.04	0.08	**0.76**	−0.52
(1) *Bedtime* (clock time in min)	−0.07	0.00	−0.04	**0.68**	−0.49
(15) *Sleep Period* (min)	0.11	0.03	−0.01	0.12	**0.99**
(13) *Sleep Opportunity* (min)	0.18	0.00	0.05	0.08	**0.96**
(17) *Total Sleep Time* (min)	−0.40	−0.03	0.04	0.06	**0.78**
Proportion of Variance explained	**0.19**	**0.14**	**0.14**	**0.13**	**0.11**

Each subsequent model was run with all 100 imputations of the PCA-derived scores for each participant and sleep composite (unweighted average of the highest loadings). All results were pooled across all 100 models. To evaluate the effects of age and sex, we used linear regression models. With the corrplot package we examined correlations between sleep composites and assessment time points using Spearman correlation coefficients. Bonferroni correction was applied to address multiple comparison issues. To test the stability of effects across development, the range of each infant's percentiles across all assessment timepoints was evaluated (within-subject stability). The stability of composites vs. single variables was evaluated using paired *t*-tests.

Associations of sleep composites with behavioral outcomes were identified based on longitudinal multilevel models using the lme4 package and by including participant ID for the intercepts and timepoint as slope. Covariates were exact age and sex, and predictors were the 5 sleep composites. Values were considered outliers if they exceeded 1.5 times the interquartile range below the 1st quartile or above the 3rd quartile. Reported statistics include outliers, but any changes in significance due to exclusions of outliers are mentioned specifically. Significance level was set to below 0.05.

3. Results

3.1. Five Principal Components Express All Infant Sleep Variables: Infant Sleep Composites

We achieved reduction of complexity of infant sleep variables by determining five core sleep composites. The relationship of each of the 48 original sleep variables with the sleep composites is represented as "loadings" (Table 2). The five sleep composites explain a total of 71% of the variances, which yields a diagonal fit of 0.98. This revealed:

- *Sleep Activity*—Larger values reflect more movements and more awakenings during the night as well as less regularity of awakenings. The most representative (i.e., with highest loading) single variable were *Sleep Efficiency* (negative) or *Longest Nocturnal Wake* (positive);
- *Sleep Timing*—Larger values reflect later clock time of bed times and sleep times. The most representative single variable was *Sleep Offset*;
- *Sleep Night*—Larger values reflect longer nighttime sleep opportunity and longer nighttime sleep duration. The most representative single variable was *Sleep Period*;
- *Sleep Day*—Larger values reflect longer daytime sleep duration, more daytime naps, and lower regularity in daytime sleep. The most representative variables were *Longest Wake* (negatively) or *Nap Counter* (positively);
- *Sleep Variability*—Larger values reflect higher variability between measurement days (standard deviation) within *Sleep Timing* and *Sleep Night*. The most representative single variable was *Variability of Sleep Opportunity*.

Interestingly *24 h Sleep Duration* showed the highest loading on the *Sleep Day* composite, meaning it was more related to *Sleep Day* than *Sleep Night*. This finding demonstrates a tight link between naps and total sleep duration. However, to make the interpretation of the *Sleep Day* composite easier, we removed this variable from subsequent analyses.

3.2. Sleep Composites Accurately Reflect Sleep Maturation Across Infancy

To ensure that the sleep composites accurately reflect the maturation of sleep patterns in infancy, we examined changes in the sleep composites across age. As expected, *Sleep Activity*, *Sleep Day*, *Sleep Timing*, and *Sleep Variability* all decreased with age (*Sleep Activity* $t_{(434.25)} = -14.59$, $p < 0.001$, *Sleep Day* $t_{(413.53)} = -25.09$, $p < 0.001$, *Sleep Timing* $t_{(426.65)} = -5.78$, $p < 0.001$, and *Sleep Variability* $t_{(423.45)} = -6.13$, $p < 0.001$). In other words, in comparison to infants at a younger age, older infants showed lower activity at night and woke up less frequently (b = −0.15 per month older), slept less often and also shorter during the day (b = −0.21), went to sleep earlier at night and woke up earlier in the morning (b = −0.07), and were more consistent in their sleep timing and nighttime sleep duration (b = −0.08). *Sleep Night* on the other hand, slightly increased with age ($t_{(421.30)} = 2.59$, $p = 0.01$), indicating that older infants slept more at night (b = 0.03). Therefore, sleep composites capture the sleep maturation in infancy well. Moreover, within the same models we could observe sex differences in *Sleep Activity* (female vs. male $t_{(431.04)} = -3.84$, $p < 0.001$) and *Sleep Variability* ($t_{(434.79)} = -1.88$, $p = 0.06$), yet the latter was significant only after the exclusion of outliers ($t_{(413.77)} = -2.21$, $p = 0.03$). Girls showed lower nightly activity and reduced wakings (b = −0.29 for female) and were more consistent in their sleep routine (with outliers b = −0.17/without outliers b = −0.19 for female). No sex differences were detected in the other sleep composites ($p > 0.05$).

3.3. Strong Correlations between the Sleep Composites

Next, we investigated the interrelationships between the sleep composites. Notably, each sleep composite correlated significantly with all other sleep composites, indicating that while sleep is a multidimensional construct, the different dimensions are tightly intertwined (all $p < 0.001$; Figure 1). Interestingly, the strongest positive correlation was found between *Sleep Activity* and *Sleep Day* ($r_s = 0.50$, $p < 0.001$). Higher activity at night was associated with more sleep during the day. Surprisingly, this

association was stronger than the association of *Sleep Activity* and *Sleep Night*. As expected, a strong positive correlation was found between *Sleep Timing* and *Sleep Variability* ($r_s = 0.48$, $p < 0.001$), i.e., the later the sleep timing, the higher the *Sleep Variability*. Stronger negative correlations were found between *Sleep Day* and *Sleep Night* ($r_s = -0.26$, $p < 0.001$) with infants that slept more during the day, slept less at night. A strong negative association was also found for *Sleep Night* with *Sleep Timing* ($r_s = -0.25$, $p < 0.001$), such that infants with later sleep times had less nighttime sleep. In summary, even though the approach clearly identified five core sleep composites of infant sleep, those composites are nonetheless also correlated with each other.

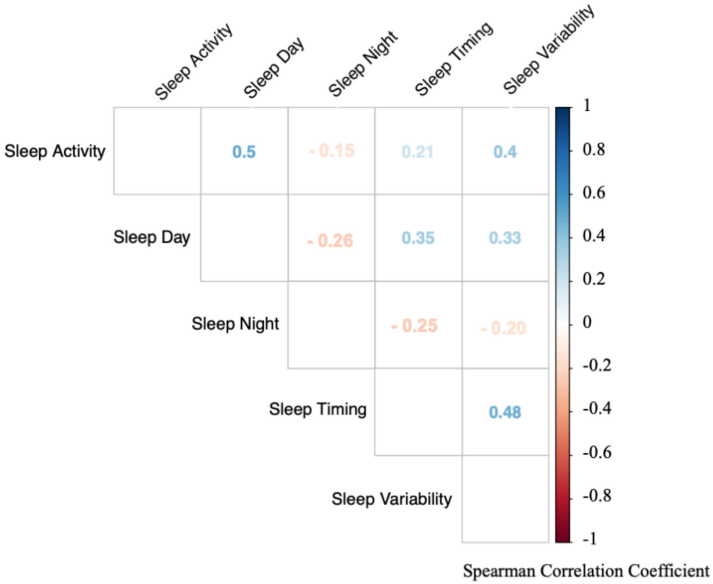

Figure 1. Correlations between the infant sleep composites based on all assessment timepoints. Each sleep composite is significantly associated with all other composites (all $p < 0.001$). Colors indicate strength of correlation (red = negative correlations, blue = positive correlations). Numbers indicate spearman correlation coefficient (r_s).

3.4. Stability of Sleep Composites

To investigate the stability of sleep composites across the first infant year, we examined correlation coefficients between all assessment time points of each sleep composite (Table 3). Most sleep composites significantly correlated between the adjacent time points (3 vs. 6 or 6 vs. 12 months). Only *Sleep Timing* also significantly correlated between 3 and 12 months. While *Sleep Variability* and *Sleep Night* significantly correlated when outliers were removed, this correlation being low and suggesting no stability ($R^2 = 0.07$). To better understand the dynamics, we calculated the within-subject stability, i.e., consistency of the position of each subject in relation to all other participants. On average, children had a maximum change of 29% for *Sleep Timing*, 38% for *Sleep Night*, 43% for *Sleep Variability* and *Sleep Day*, and 45% for *Sleep Activity* from 3–12 months (values from one imputation). This suggests that although most sleep behaviors are stable in the short term, they are dynamic across the first year of infancy.

Table 3. Spearman Correlation Coefficients (r_s) of sleep composites across assessment time points. Significant correlations are presented in bold (Bonferroni corrected *p*-value below 0.0033). The correlation marked with * are significant upon exclusion of outliers: *Sleep Variability* $r_s = 0.26$, $p = 0.002$, *Sleep Night* $r_s = 0.26$, $p = 0.001$). Composites are most stable across adjacent time points, but only *Sleep Timing* is stable across the entire first year.

Sleep Composite	Correlation 3 vs. 6 Months		Correlation 6 vs. 12 Months		Correlation 3 vs. 12 Months	
	r_s	*p*	r_s	*p*	r_s	*p*
Sleep Activity	**0.29**	**<0.001**	0.21	0.01	0.15	0.07
Sleep Day	0.25	0.004	**0.29**	**<0.001**	0.11	0.21
Sleep Night	**0.53**	**<0.001**	**0.45**	**<0.001**	0.24 *	0.004
Sleep Timing	**0.68**	**<0.001**	**0.58**	**<0.001**	**0.55**	**<0.001**
Sleep Variability	**0.28**	**<0.001**	**0.38**	**<0.001**	0.23 *	0.007

3.5. Stability of Sleep Composites vs. Single Sleep Variables

Subsequently we tested whether the sleep composites were more stable across the assessment timepoints compared to the stability of single sleep variables, as observed in young children and adults [27]. We used the within-subject stability and compared it between single and composite variables. We tested this within-subject stability in *Sleep Timing* (the most stable variable) and in *Sleep Activity*, (the least stable variable). Within-subject stability was also computed for the single sleep variables that loaded the highest and lowest on both sleep composites: *Sleep Offset* and *Bedtime* as well as *Longest Nocturnal Wake* and *Percent Active Epochs*. An exemplary comparison of one imputation and six random participants is shown in Figure 2. There was no significant difference between the within-subject stability of *Sleep Activity* and *Longest Nocturnal Wake* ($t_{(115.69)} = -0.17$, $p = 0.86$) nor between the within-subject stability of *Sleep Activity* and *Percent Active Epochs* ($t_{(134.03)} = 0.10$, $p = 0.92$), indicating no advantage in within-subject stability in the sleep composite as compared to within-subject stability in single sleep variables. In other words, infants showed variable sleep behavior no matter how it was quantified. Similarly, there was no significant difference between the within-subject stability of *Sleep Timing* and *Bedtime* ($t_{(124.88)} = 0.40$, $p = 0.69$). Contrastingly, *Sleep Timing* showed higher within-subject stability as compared to *Sleep Offset* ($t_{(106.64)} = 3.20$, $p = 0.002$). Thus, we cannot confirm higher within-subject stability in sleep composites compared to within-subject stability in single sleep variables across the first year of life.

3.6. Association of Sleep Composite with Behavioral Development

Lastly, we evaluated whether infant sleep composites are linked to behavioral developmental status. Multilevel models across all assessment timepoints revealed a negative link between *Sleep Day* and ASQ-*Collective score* (b = −6.65, $t_{(344.65)} = -2.22$, $p = 0.03$). No association was observed between behavioral development and other sleep composites ($p > 0.05$, Table 4). The effect between *Sleep Day* and *Collective Score* was more pronounced after reducing the model to only include *Sleep Day* and control variables (exact age, sex) and no other sleep composites (b = −7.88, $t_{(358.69)} = -2.83$, $p = 0.005$). This association suggests that infants with more daytime sleep had lower overall developmental scores. To investigate this finding in more depth we determined whether the effects persisted in the two behavioral sub-scores *Personal-social* and *Gross Motor*. This was not the case as no significant effects between the behavioral sub-scores and any of the sleep composites were found ($p > 0.05$). It is thus likely that the effect of *Sleep Day* with behavioral developmental is driven by the combination over multiple scales of development.

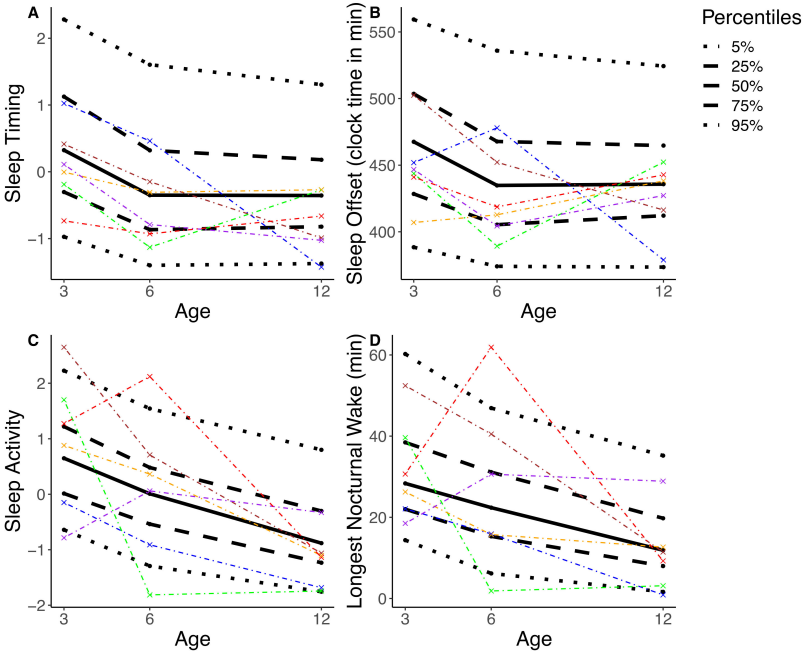

Figure 2. The percentile distribution is illustrated based on one randomly selected imputation (black; solid line = median, dashed line = interquartile range, dotted line = 90th percentile). A total of six randomly selected participants are represented, each with specific color. (**A**) shows *Sleep Timing*, the most stable composite. (**B**) shows *Sleep Offset*, the highest loading single variable for *Sleep Timing*. (**C**) shows *Sleep Activity*, the least stable composite. (**D**) shows *Longest Nocturnal Wake*, the highest loading single variable for *Sleep Activity*. Results illustrate that the position of a participant within the percentile distribution fluctuates across the assessments of 3, 6, and 12 months. In other words, e.g., an infant with a comparatively high score on *Sleep Activity* at 3 months does not necessarily maintain a high score in *Sleep Activity* at 6 and 12 months.

Table 4. Associations between sleep composites and behavioral development as quantified by the Ages and Stages questionnaire. Bold font indicates significant associations ($p < 0.05$). SE = Standard error of measurement.

Variable	*Collective Score*		*Personal-Social*		*Gross Motor*	
	b ± SE	***p***	**b ± SE**	***p***	**b ± SE**	***p***
Intercept	203.16 ± 6.58	<0.001	42.17 ± 2.04	<0.001	38.82 ± 2.21	<0.001
Sleep Activity	−0.91 ± 2.27	0.69	−0.46 ± 0.73	0.53	1.05 ± 0.80	0.19
Sleep Day	**−6.65± 3.00**	**0.03**	−1.08 ± 0.98	0.27	1.12 ± 1.10	0.31
Sleep Night	0.84 ± 2.08	0.68	0.49 ± 0.62	0.43	0.68 ± 0.69	0.33
Sleep Timing	−0.20 ± 2.41	0.94	−0.40 ± 0.70	0.57	0.47 ± 0.78	0.55
Sleep Variability	−2.52 ± 2.09	0.23	0.33 ± 0.67	0.62	0.55 ± 0.77	0.47
Exact age	0.21 ± 0.79	0.78	−0.37 ± 0.27	0.17	0.14 ± 0.30	0.63
Female sex	9.49 ± 5.63	0.09	1.76 ± 1.35	0.19	0.90 ± 1.55	0.56

4. Discussion

In this study we demonstrate that numerous dimensions of infant sleep can be reduced to the five core sleep composites: *Sleep Activity*, *Sleep Timing*, *Sleep Day*, *Sleep Night*, and *Sleep Variability*. The sleep composites undergo developmental changes that align with the known maturation of sleep behaviors. We thus recommend the use of sleep composites to reduce variables and to streamline analyses between different lines of research.

Furthermore, both the majority of sleep composites, as well as the single sleep variables show only limited within-subject stability across the first year of infancy, which contrasts with reports in older children. The only notable exception is *Sleep Timing*, which is stable across the first year of life, and indicates either a parental or infant preference. The lack of within-subject stability can be problematic for studies with a single assessment time-point, because results will vary depending on the assessment time-point. We thus recommend the use of multiple assessment time points, especially when the early sleep behavior is used to predict later cognitive or behavioral outcomes. Interestingly, *Sleep Day* is associated with behavioral developmental scores, therefore being a potential marker for maturation. Additionally, we report a sex difference in *Sleep Activity*, with male participants showing more and longer awakenings during the night compared to female infants.

We confirm for the first time the existence of the five infant sleep composites, *Sleep Activity*, *Sleep Timing*, *Sleep Day*, *Sleep Night*, and *Sleep Variability*, as previously identified in 2.5–3.5-year-old children [27] and which correspond to the most fundamental dimensions of sleep regulation. We adhered to the same terminology used by Staples et al., except for replacing *Sleep Duration* with *Sleep Night* to differentiate it from *Sleep Day*. To represent sleep in the earliest period of life, we included several variables pertaining to daytime sleep (e.g., number of naps, longest duration of consolidated wake). This confirmed *Sleep Day* as a construct separate from the other sleep composites. In comparison to Staples et al. our method explains sleep variable variance to a slightly lower extent (71% vs. 82%), which might be caused by the difference in the assessed sleep variables or the different age range (33 vs. 18). Importantly, the proposed sleep composites follow the primary developmental trajectories of the single sleep variables [17,61]. Overall, we conclude that the sleep composites are consistent from infancy to childhood and correspond well to the known core maturation of infant sleep patterns.

The selection of variables can be difficult because of the diversity of sleep variables and computations. Using too many variables can lead to multiple testing problems and increase false positive findings [26]. Thus, using composites to reduce the number of variables facilitates investigations in multi-dimensional research. Our results demonstrate that the resulting sleep composites remain consistent across early development, which aligns with Staples et al., even though different sleep variables were used for computations and even though actigraphy devices differed (GENEActive in our study, MicroMini Motionlogger used by Staples et al.). While in both studies a Sadeh algorithm was used, we used the algorithm specifically developed for infants [33] whereas Staples et al. used the Sadeh algorithm developed on adolescents and adults [62] and then validated in children [28]. Our analysis confirms that all single sleep variables identical to Staples et al., [27] loaded onto the same sleep composite. This strongly supports the use of sleep composites, which has the additional advantage to enhance comparability across studies. Moreover, when computation of sleep composites is not possible, our results can guide the selection of variables. Specifically, it is preferable to select single sleep variables to reflect all sleep composites.

While sleep composites showed some stability across adjacent time periods (3–6 and 6–12 months), the majority of sleep composites did not maintain strong stability across the longest period from 3 to 12 months (except for *Sleep Timing*). This aligns with a previous report, which examined stability of sleep behaviors from 3 to 42 months [11] and found more stability in sleep duration across shorter time intervals while sleep onset time was very stable. Compared to children, adolescents, and adults, the stability of sleep variables is exceptionally low in infants. In children 3–7 years old, the year-to-year stability was moderate (r = 0.4–0.6) in variables related to *Sleep Night* and *Sleep Timing* (even though low stability was noted in *Sleep Activity*) [63]. Thus, the stability of *Sleep Night* increases until childhood,

while *Sleep Timing* remains stable and *Sleep Activity* remains variable until adolescence. A 10-year-long study examining dynamics of sleep duration based on interviews from ages 1 to 10 years reported annual fluctuations, yet overall long-term stability [64]. In adults, year-to-year correlation is high for most sleep measures, especially when derived from several nights (r = 0.48–0.93) [65–68]. While in older children, the instability of sleep behaviors might be due to measurement imprecisions and therefore is improved by using composites (shown by Staples et al.), it seems that the instability of sleep behaviors in infancy is inherent in the behavior itself. Hence, because variability in infant sleep persists naturally, infant sleep composites are not eliminating this variability. One solution to address this point, specifically when examining later outcomes, is to perform a repeated-measures design, as has been previously suggested by Ednick et al. [69]. If this is not possible, it is important to clarify the age group a finding relates to.

The high within-infant stability of *Sleep Timing* is notable and we assume that it is largely parent-driven. This is confirmed by the finding that parent's bedtimes are positively correlated with *Sleep Timing* (Mother $r_s = 0.33$, $p < 0.001$ Father $r_s = 0.24$, $p < 0.001$; exploratory analysis using the reported bedtimes in the Pittsburgh Sleep Quality index). Not surprisingly, parents with later bedtimes also have infants with later sleep timing. However, interestingly, parental bedtimes only explain a small amount of variance in *Sleep Timing* (Mother partial $\eta^2 = 0.05$, Father partial $\eta^2 = 0.03$). Therefore, variance in infant's sleep remains unexplained by parent's bedtime preferences. It is unclear whether this variance relates to other parental factors (e.g., cognitions about regular timing of infant sleep), or if the infants themselves already start to demonstrate clock time preference as an early form of infant chronotype. It has previously been reported that infant chronotype may depend on the infant's sex [70]. However, we did not find any differences in *Sleep Timing* between boys and girls, which would support this concept.

Intriguingly, we found a difference in *Sleep Activity* between male and female infants. This is both surprising because most previous studies in infants reported no sex differences [11,71,72] and unsurprisingly, because these differences are well known in adults [73–75]. Boys commonly show higher activity levels [76], which could cause more activity during sleep in infant boys. However, one study reported sex differences as young as 2 weeks in electroencephalographic recordings [77]. Therefore, with methods that are sufficiently sensitive, sex differences in sleep behaviors can be detected already very early in life.

A final study goal was to examine if any of the sleep composites mirrors behavioral maturation. Thus, we tested the association between sleep composites and behavioral developmental status. Indeed, infants with more daytime sleep (*Sleep Day*) had lower ASQ-Collective scores. Our results align with Spruyt et al. who report a negative association between daytime sleep at 12 months with emotional regulation and behavioral maturation [78]. Of relevance might be that the variable *Sleep Day* shows the largest developmental changes across the first year of life. For example, the infant's hours asleep during the day as well as the number of naps are dramatically reduced by half in the short period from 3 to 12 months of age. Furthermore, the neurophysiology of daytime sleep also changes with age: 5-year-old children show decreased slow wave activity (a marker of sleep need) during an afternoon nap compared to 2- and 3-year-old children [10], which also suggests that daytime sleep specifically reflects maturation of the central nervous system. When infants are young, napping is important for new memory formation [5,79,80]. When infants get older, their tolerance of longer wake periods increases, which likely also includes their capacity of information acquisition without an immediate nap. Therefore, we hypothesize that a faster decrease in daytime sleep reflects more advanced maturation on a neuronal level. Kurdziel et al. support this theory by demonstrating that naps enhance memory performance in pre-school children only in those children who habitually nap [81] (however see [82]). Therefore, children likely stop to take regular naps when they have developed a physiological tolerance to longer wake periods and when they can retain information without a subsequent nap.

Limitations

We included a comprehensive list of commonly used sleep variables of infants and young children. Since the structuring of factors in the principal component analysis depends on the variables used, the sleep composites identified in this study might not be representative for other investigations that include different single sleep variables. Furthermore, the choice of five factors for the PCA was based on both, data driven criteria, as well as on the interpretability of the resulting sleep composites. It is therefore possible that from a data-driven perspective, more factors would result in a better model fit. However, we prioritized the interpretability of composites so that infant sleep composites can be used for analysis with other datasets. Furthermore, our data are biased toward a higher parental education level (data not shown) and therefore more homogenous than the general population.

5. Conclusions

Our five sleep composites accurately characterized the complex dimensions of infant sleep and reflect known maturational dynamics of infant sleep. To increase comparison across studies, we suggest that researchers use infant sleep composites or, if not possible, single sleep variables with high loadings on the sleep composite of interest. As infant sleep behavior is highly variable both between and within infants, we recommend using multiple assessment time points, especially for testing sleep behaviors as predictors for later cognitive, emotional, or behavioral outcomes. Future experiments may target *Sleep Timing* as a possible early chronotype and *Sleep Day* as a maturational marker. Therefore, this study opens up new possibilities to standardize and advance the emerging field of infant sleep research.

Supplementary Materials: The following are available online at http://www.mdpi.com/1424-8220/20/24/7188/s1, Table S1: Exclusion Sleep Variables, Table S2: Prediction Matrix. The datasets are available from the corresponding author on reasonable request and pending ethics approval. The analysis scripts are deposited on the Open Science Framework: DOI 10.17605/OSF.IO/6S3PW.

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Appendix A

The imputation data set included the participant number (complete), the timepoint of assessment (complete), gender (complete), exact age at assessment (complete or set to assessment timepoint for data points to impute), *Collective Score* (4.5% missing), *Gross Motor* (4.5 % missing), and *Personal Social* (4.5% missing) scores from the Ages and Stages questionnaire [29], gestation age at birth (complete), sleep environment for the baby at night (own room, parent's room, room shared with sibling; complete for missing data we inferred from the other assessment time points), number of children in family (complete), analysis run for gut microbiota (complete, if there was not gut microbiota data, the run

at which the data would have been analyzed was used), probiotics use (yes/no) at 3 (5.2 % missing) and 6 months (3% missing), antibiotics use at 6 (3% missing) and 12 months (6.7% missing, "never", "unknown time", "2–4 weeks before assessment", "<2 weeks before assessment"), 3 alpha diversity measures for the gut microbiota (Shannon, Observed, Chao all 4.1% missing), gut microbiota cluster (4.1% missing), gut microbiota age prediction (4.1% missing), education mother (0.2% missing) and father (2.8% missing, "none", "apprenticeship", "high school", "university", "PhD"), and bottle and breastfeeding frequency (4.9% missing, 0 for never or rarely breastfed, 1 if breastfed occasionally, regularly or daily). All 48 sleep variables were included, which ranged from 4.9% missing (*Bedtime*) to 22.3% missing (*Variability of Total Sleep Time*, *Variability of Sleep Efficiency*, *Variability of Sleep after Wake Onset*). The missing data from each variable were predicted by all other variables in the dataset that correlated with the variable with $r \geq 0.1$. The parental education and bottle and breastfeeding frequency variables had to be excluded as predictors because inclusion of them lead to inability to specify the model (see prediction matrix in the Supplementary Table S2).

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6.4 ARTICLE 4

From Alpha Diversity to ZZZ: Exploring associations among sleep, gut bacteria and behavioral development in infancy

Sarah F. Schoch, Josue L. Castro-Meija, Dennis S. Nielsen, Lukas Krych, Witold Kot, Bingfeng Leng, Malcolm Kohler, Reto Huber, Gerhard Rogler, Luc Biedermann, Jean-Claude Walser & Salome Kurth

Abstract

Infancy is a period of marked development, entailing many maturation processes that lay the foundation for later health. Two crucial health factors in infancy are the establishment of a sleep-wake rhythm and the growth of gut bacterial diversity - both have been linked to later cognitive and physiological outcomes. In adults and animal models, evidence is emerging that sleep and gut bacteria are bidirectionally linked. However, it is yet unclear when and how the sleep-gut link in early life evolves. Here we demonstrate that a sleep-gut link develops in infancy, undergoes transitional dynamics, and involves an early sensitive period. Specifically, infants' daytime sleep is linked to markers of bacterial diversity, and nighttime awakenings are associated with gut bacterial maturity and bacterial enterotype. Furthermore, both sleep and gut bacteria are associated with behavioral developmental outcomes, with the strongest connections at 3 months of age. Our results demonstrate the dynamic interplay of sleep and gut bacteria within healthy human development. As both sleep and gut bacteria are modifiable, they are promising targets for early intervention in clinical groups.

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From Alpha Diversity to ZZZ: Exploring associations among sleep, gut bacteria and behavioral development in infancy

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Abstract

Infancy is a period of marked development, entailing many maturation processes that lay the foundation for later health. Two crucial health factors in infancy are the establishment of a sleep-wake rhythm and the growth of gut bacterial diversity - both have been linked to later cognitive and physiological outcomes. In adults and animal models, evidence is emerging that sleep and gut bacteria are bidirectionally linked. However, it is yet unclear when and how the sleep-gut link in early life evolves. Here we demonstrate that a sleep-gut link develops in infancy, undergoes transitional dynamics, and involves an early sensitive period. Specifically, infants' daytime sleep is linked to markers of bacterial diversity, and nighttime awakenings are associated with gut bacterial maturity and bacterial enterotype. Furthermore, both sleep and gut bacteria are associated with behavioral developmental outcomes, with the strongest connections at 3 months of age. Our results demonstrate the dynamic interplay of sleep and gut bacteria within healthy human development. As both sleep and gut bacteria are modifiable, they are promising targets for early intervention in clinical groups.

Introduction

One in six children is affected by a developmental disorder (e.g., autism, developmental delays) [1]. Early recognition of such disorders is crucial to provide effective support and prevent aggravated consequences [2]. Two potential health targets in early childhood that can serve as protective factors are sleep behavior and gut bacteria.

Sleep regulation undergoes significant maturation in early development, with the most drastic changes in the first year of life. Although sleep behavior is highly variable between infants, universal maturational patterns are visible: in the first year of infancy, a 24-h rhythm emerges, and nighttime sleep is consolidated [3,4]. In the past decade, empirical work has revolutionized our understanding: from sleep as a global behavior towards sleep as a localized neurophysiological and cellular recovery process that interacts with neurodevelopment [5,6]. Sleep neurophysiology is assessed with the electroencephalogram (EEG) and unfolds throughout early childhood. This evolution entails not only a pronounced decrease of the fraction of rapid eye movement (REM) sleep [7] but, furthermore, the emergence of core sleep features such as sleep spindles [8–10] and slow waves [11,12]. Sleep and brain development are closely linked: slow waves mirror the underlying brain maturation processes [13–15]. Excitingly, the sleep-brain link goes beyond pure correlative observations: short, fragmented, or poorly consolidated sleep in infancy predicts later cognitive and psychosocial problems [16–19] (but see [20–22]), supporting now in young humans the demonstrated concept that the formation of neuronal connections necessitates adequate sleep [5,23–25].

Beyond sleep, we have come to understand the influence gut bacteria exert on the brain (for a comprehensive review, see [26]). Seminal research with germ-free mice, which lack this microbial ecosystem in the digestive tract, illuminates the bottom-up processes through which gut bacteria affect the brain [27–29]. Newly emerging insights on the gut-brain axis illuminate gut bacterial influences [30], how they promote stress regulation and mental health [31–33].

Early bacterial colonization is crucial because it is likely connected with the initiation of signaling processes in the brain. For example, germ-free mice have an

exaggerated stress response compared to the control group of specific pathogen-free mice. Interestingly, the restoration of gut bacteria can partly reverse the stress response - yet exclusively in young but not in adult germ-free mice [34,35]. In alignment, compelling evidence is now emerging that gut bacteria also play a pivotal role in human brain development: The composition and diversity of bacterial taxa in infants' gut - quantified from stool samples - are linked to physiological growth and cognitive outcomes later in life [36]. Preliminary correlates between individual infant gut bacterial profiles with regional gray matter maturation support the gut-brain concept in infancy [37]. However, it remains unclear if and how sleep and gut bacteria jointly act throughout the process of neurodevelopment.

In adult humans, specific gut bacteria profiles have been associated with sleep disturbances, sleep quality, and sleep duration [38–40]. Both the timing and the duration of sleep influence gut bacteria [41–49] (yet [50]). In rodents, experimentally depleting gut bacteria specifically affects slow-wave sleep [51] - the most established proxy of sleep depth, and at the same time, the most conceptualized driver of neurodevelopment [13,52]. Crucially, a large fraction of gut bacterial taxa undergoes circadian rhythmicity influenced by the host circadian rhythm [53–55].

Interestingly, probiotics intake alleviated subjective and objective sleep disturbances related to stress in humans [56]. The sleep-gut-link may, therefore, be bi-directional. In strong support of this idea, experimental alterations of gut bacteria were shown to modify sleep consolidation in mice and rats [57–59].

It is unclear when the sleep-gut link starts to develop and when its maturation is completed. Yet, it is likely that a sleep-gut-brain link exists already in infancy, considering the sleep-neurodevelopment link, the abovementioned gut-brain axis together with the new evidence for a sleep-gut-brain link from investigations in adult humans and rodents. Importantly, the sequence of sensitive periods for brain development may pinpoint the sleep-gut link to be most pronounced, when the brain is most susceptible to external influences and thus most plastic in its connectivity. The early establishment of the sleep-gut link may therefore indeed determine later behavioral-developmental outcomes.

This study examines whether a sleep-gut link exists in infancy and how its dynamics evolve across the first year of life. We hypothesized that more mature sleep-wake behavior patterns are linked to more mature infant gut bacteria profiles, the latter represented by the three measures: gut bacterial diversity, bacterial maturation index, and the assignment to enterotypes. To capture sleep maturation in great detail, we included the five primary infant sleep composites (*Sleep Activity*, *Sleep Timing, Sleep Night*, *Sleep Day*, and *Sleep Variability*) aligned with the sleep composites reported by Staples et al. (2019) [60]. Additionally, we expected a positive predictive effect of early maturity of sleep patterns and gut bacteria on later behavioral developmental status.

METHODS

Participants

162 healthy Swiss infants (75 female) participated in a study in which the three domains sleep, gut bacteria, and behavioral development were longitudinally investigated. We recruited infant participants with good general health, who were primarily breastfed, vaginally and term-born (37 – 43 week gestation week), and who experienced no antibiotic intake in their first three months of life (for detailed inclusion and exclusion criteria see [4]). Sub-sections of this dataset were published previously [4,61]. Ethical approval was obtained from the *cantonal ethics committee* (BASEC 2016-00730), and study procedures were consistent with the declaration of Helsinki. Written parental consent was obtained after explanation of the study and before enrollment. Families received small non-monetary gifts for their participation.

Experimental design

At infant age 3, 6, and 12 months, we quantified the three domains age-appropriately with validated objective and subjective methods (see Figure 1). At age 24 months, we conducted a follow-up assessment of behavioral development.

We measured infant sleep with ankle actigraphy and a 24-h-diary [61,62] for 11 continuous days. Parents attached the GENEactiv movement sensors (Activinsights Ltd, Kimbolton, UK, 43x40x13 mm, MEMS sensor, 16 g, 30 Hz Frequency) on the

infants' left ankle using a modified sock or a Tyvek paper strap. We instructed parents to only remove the actigraph for bathing/swimming activities and document actigraph removal in the 24-h diary.

During each of the three assessments, parents collected at least one stool sample from the infant's diaper using disposable pipettes (Pastette 3 ml graduated) or disposable laboratory spatulas (smartSpatula, 210 mm natural). Samples were kept in sterile Eppendorf tubes (5 ml), wrapped into Whirlpak bags, and temporarily stored in the families' fridge. Samples were then transported to the laboratory within 48 h in cooling boxes to maintain temperature (n = 19 samples were transported within 72 h). Aliquots with a minimum weight of 100 mg were sampled and stored at -50° Celsius.

Infant behavioral developmental status was quantified with a parent-completed German translation of the Ages and Stages Questionnaire [63]. Using online questionnaires, we assessed feeding practices, sleep habits, general health, and demographics.

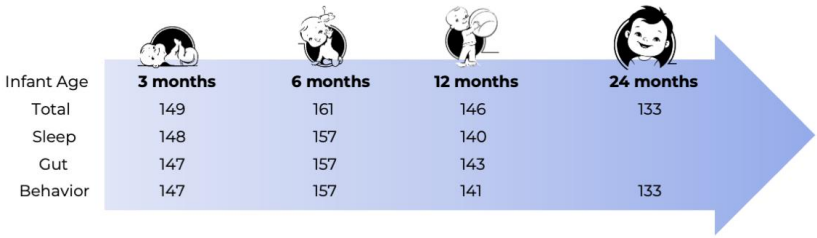

Figure 1. Experimental design to investigate the longitudinal interrelationships of sleep, gut bacteria, and behavioral development in healthy infants. Sleep entailed the collection of actigraphy and 24-h diary data [61], gut bacteria were characterized with 16S rRNA analysis from fecal samples, and behavior was quantified with the parent-reported Ages and Stages Questionnaire [63]. The sample size is indicated for each assessment in the corresponding domain. Graphics from Natalia Zelenina © 123RF.com.

Missing Data

10 infants were exclusively recruited for the 6 months assessment. Additionally, some infants were excluded for single assessment time points (n = 2 at 3 months due to medication intake, n = 1 at 6 months due to health reasons and n = 6 at 12 months due to participant attrition and illness). Furthermore, 11 infants lacked sleep data for single assessment time points (n = 1 at 3 months due to holidays, n = 4 at 6 months due to device failure (3) and withdrawal from sleep data collection (1), n = 6 at 12 months due to device failure (2), moving away (1), prolonged illness (1), and withdrawal from sleep data collection (2)). Gut bacteria data was lacking from 9 infants for single assessment time points (n = 2 at 3 months due to low read count (1) and holidays (1), n = 4 at 6 months due to low read count, n = 3 at 12 months due to moving away (1), low read count (1) and sample not analyzed (1)). Behavioral data was missing from 11 infants for single assessment time points (n = 2 at 3 months and n = 4 at 6 months and n = 5 at 12 months of age all due to completion of questionnaire at an age outside of the selected time window). Additionally, only single recording days of sleep data were missing [4] or single items were not marked in survey. Missing data was replaced with 100 imputations using the R package *mice* [4] for sleep as well as gut bacteria outcome (i.e., alpha diversity, enterotype, random forest classification – methodological details follow), yet not for the intermediate quantification of gut bacterial counts.

SLEEP

Actigraphy analysis

Actigraph data were processed according to our laboratory standards [61]. We computed infant sleep-wake patterns with a 6-step modification of the Sadeh algorithm [4,61,64]. We calculated 48 sleep variables of interest, which were based on the recommendation of Meltzer et al. (2012) and Staples et al. (2019), and added a sleep regularity index [60,65,66]. To comprehensively capture all aspects of infant sleep maturation and at the same time reducing the number of analyses, we computed “sleep composites” [4]. A principal component analysis implemented a data-driven, similarity-based way: the 32 of the 48 sleep variables were consolidated into five sleep composites, which ultimately contained the key dimensions of infant sleep:

- *Sleep Activity*, summarizing movement and awakenings at night,

- *Sleep Timing,* representing the clock time of bedtimes and sleep times,
- *Sleep Night*, reflecting nighttime sleep opportunity and duration,
- *Sleep Day*, characterizing duration and number of daytime naps and their regularity, and
- *Sleep Variability*, reflecting the variability of timing and nighttime sleep between the recorded days.

GUT BACTERIA

Stool DNA extraction and 16s rRNA-gene amplicon sequencing

DNA was extracted from ~200 mg of stool using PowerSoil® DNA Isolation Kit (MOBIO Laboratories, Carlsbad, CA, USA) and following the manufacturer's instructions, but with minor modifications. Before DNA extraction, samples were placed into the PowerBead tubes and heat-treated at 65°C for 10 min and then at 95°C for 10 min. Subsequently, solution C1 was added, and bead-beating was performed in FastPrep (MP Biomedicals, Santa Ana, CA, USA) using three cycles of 15 s each, at a speed of 6.5 m s^{-1}. The remaining DNA extraction procedure followed the manufacturer's instructions.

NextSeq-based 16S rRNA gene amplicons were sequenced from the V3 region [67] using primers designed with adapters for the Nextera Index Kit® (Illumina, CA, USA): NXt_338_F: 5'- TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG ACW CCT ACG GGW GGC AGC AG -3' and NXt_518_R: 5'- GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GAT TAC CGC GGC TGC TGG -3'. Amplification profile (1st PCR), barcoding (2nd PCR), amplicon library purification, and sequencing were performed as previously described [68]. The raw dataset containing 2 x 151bp (pair-ended) was quality checked using FastQC (v.0.11.2) [69]. Overlapping forward and reverse reads were trimmed (R1:5bp, R2:10bp) using seqtk [70] and merged using FLASH (v1.2.11) [71] with the following parameters: minimum overlap of 15, a maximum overlap of 300, and maximum mismatch density of 0.25. Cutadapt (v1.12) [72] was used to identify and trim primer regions allowing for an error rate of 0.01. Quality filtering was done using PrinSeq [73], removing reads with mean quality lower than 20 or containing ambiguous nucleotides. The resulting amplicons were de-noised and assigned to define Operational Taxonomic Units

(OTUs) at 97% sequence identity using usearch (v10.0.240, UNOISE3) [74]. Taxonomic predictions were made using SINTAX [75] and the Greengenes catalog [76].

Analysis of gut bacteria data

Samples were analyzed in five batches, across which the variability in sequencing depth was computed. To account for possible differences in sequencing depth and gut bacteria composition and diversity between batches, 'Batch' was included in all further analyses as a control variable. Samples below 50'000 reads (< 1% of all samples) were excluded to ensure ample sequencing depth (leading to n = 6 missing, as reported above). Data were normalized by rarefying to the lowest count number (50'268), resulting in 1'430 bacterial taxa.

Ordination plots based on bray distance were inspected for quality-check by uncovering possible sample irregularities due to mother and infant antibiotic use or sickness. Although the vast majority of these flagged samples were within the normal range, we nonetheless computed a reduced data database with exclusion of six potential outliers (sickness n=2, maternal antibiotic use n=3, infant antibiotic use n=1). Results with this reduced dataset are specifically referred to in the text.

Negative controls (water probes) also contained reads (range 2 - 150'785). ZOTU2 (Enterobacteriaceae) were the abundant OTUs in 30% of negative controls and in 10% of the effective infant stool samples. Other OTUs, which were abundantly detected in the negative controls, were rare in the effective samples. The influence of ZOTU2 on the three bacterial markers (gut bacterial diversity, bacterial maturation index, enterotype) was negligible.

We defined the core bacteria by the fulfilling of two criteria across all time points: bacterial taxa reaching a minimal prevalence of 20% (i.e., detected in at least 20% of infants) and a minimal relative abundance of 1% (i.e., accounting for at least 1% of all bacterial measured in total). Following this preprocessing, we used three markers to streamline the quantification of gut bacterial profiles:

i) Gut bacterial diversity: characterizing the presence and abundance of bacterial taxa within individual samples (alpha diversity),

ii) Bacterial maturation index: reflecting the relative maturational status of each individuals' gut bacterial profile computed through the difference

between random forest prediction of chronological age and actual chronological age,

iii) "Enterotypes": classifying individuals' gut bacterial profiles and assignment to groups with similar typical bacterial representatives.

For i), we computed three measures to thoroughly capture alpha diversity: Observed, Shannon, and Chao1. Observed emphasizes the number of different bacterial species present in a sample; Shannon concentrates on both - species abundance and evenness (similarity of abundance between species), and Chao1 is an abundance estimator with a focus on rare species [77].

For ii), random forest analysis was performed to predict chronological age from the bacterial composition in each stool profile (*predicted bacterial age*) [78]. We aimed to identify those infants demonstrating a mismatch between *actual* and *predicted bacterial age*. Therefore, we selected the comparatively low number of 100 trees in random forest analysis and then calculated a bacterial maturation index: "*predicted bacterial age - actual age"*.

For iii), we first applied clustering scoring methods (prediction strength, average silhouette width, and Calinksi–Harabasz scoring) to assess the ideal number of clusters (2 to 12) and distance measure (Bray-Curtis, Jaccard, Unifrac, weighted Unifrac, and Jensen-Shannon Distance) and 2 to 12 clusters [79]. Most support was found for a 2-cluster solution with weighted Unifrac. Based on this solution, each infant at each assessment time point was assigned to either enterotype A or B. Additionally, we examined the change in enterotype evolution pattern across the first year by classifying infants to "Switchers", "Bifidobacterium", "Bacteroides" and "Reverser".

BEHAVIORAL DEVELOPMENTAL STATUS

Ages and Stages Questionnaire

To quantify behavioral developmental status, parents completed a German translation of the Ages and Stages questionnaire, appropriate for the respective infant age [63]. We calculated a *Collective Score* for overall development by assembling the five key domains *Communication*, *Gross Motor*, *Fine Motor*, *Problem Solving,* and *Personal Social*. In addition to the overall *Collective Score*, we examined *Gross Motor* and *Personal Social* independently [63,80,81].

Statistical analysis

Statistical analysis was done using R Studio (1.3.959 using R version 4.0.0) with the following packages for data handling and statistics (*tidyr, eeptools, reshape, dplyr, lubridate, phyloseq, vegan, microbiome, VIM, margrittr, chron, kableExtra, knitr, lsr, reshape2, multilevel, randomForest, factoextra, cluster, fpc, mice, miceadds, lme4, nlme, data.table, stringr*) and plotting (*corrplot, ggplot2, ggpubr, lattice, ggfortify, sjPlot, cowplot, plotly, RColorBrewer, gridGraphics*) [82–116].

We ran linear models to examine alpha diversity changes with age while controlling for batch (5 different runs) and breastfeeding (0 for never or rarely breastfed, 1 for occasionally, regularly, or daily breastfeeding). To examine the batch effect on gut bacterial diversity, we also ran a one-way ANOVA to estimate h2 for batch versus age. With an Adonis test (a randomization/Monte Carlo permutation test), we examined age-related changes in beta diversity (differences between infants).
To test whether a sleep-gut link exists in infants, we applied multilevel models with the three markers for gut bacterial profiles (gut bacterial diversity, bacterial maturity index, and enterotype) as dependent variables and the five sleep composites (Sleep Day, Sleep Night, Sleep Timing, Sleep Activity, and Sleep Variability) as independent variables. Random intercepts were set for each participant. Due to the differences mentioned above, we controlled exact age, sex, batch, and breastfeeding.
Additionally, generalized linear models were computed for each assessment time point at 3, 6, and 12 months. Where enterotype was the outcome, generalized (multilevel) models with a binomial distribution were applied. Further, we examined the influence of enterotype pattern on sleep behavior at 12 months by using five generalized linear models. Each had one sleep composites at 12 months as the outcome and the enterotype evolution patterns as predictor variables (compared to the most common enterotype pattern, “Switcher”).
The five sleep composites and the three gut bacteria markers were compared to behavioral development at 3, 6, and 12 months to examine the sleep-gut-brain link. For predictive associations, behavioral outcomes at 6, 12, and 24 months were included. For predictive associations, behavioral outcomes at 6, 12, and 24 months were included. We used random intercept cross-lagged panel models [117] to separate within-person and between-person variance in longitudinal analyses by

including a latent intercept for each construct across assessment time points. The three behavioral outcomes (*Collective Score, Gross Motor Score,* and *Personal Social Score*) were tested in a model including all five sleep composites and a model including all three gut bacterial markers, resulting in 6 models in total. We controlled for exact age and sex (at the intercept level) and breastfeeding for those models with bacterial predictors. These models did not consist of imputed data but instead estimated missing values using full information maximum likelihood (FIML). FIML is a general estimate of missing predictor data, yet not of outcome. We specified the five sleep composites as latent variables using the 32 sleep variables on which the composites are based [4]. For alpha diversity, we specified a latent variable onto which the three different diversity index load. Enterotypes, bacterial maturity index and the 3 behavioral outcomes were analyzed as manifest variables.
The alpha level was set to $P < 0.05$. Results reaching significance only at trend level and results from the more restrictive version of the same dataset (outliers removed) are provided in the supplementary file.

RESULTS

Characterization of gut bacteria across infancy

Gut bacterial composition in infancy

Before addressing the primary investigation of the sleep-gut-brain link with the three gut bacterial markers, we started by characterizing the general maturation of gut bacterial profiles (Figure 2). First, we examined the abundance and prevalence of the core bacterial genera (Figure 2A). *Bifidobacterium* and *Bacteroides* were the most abundant genera across the first year of life. Infants maintained a similar overall composition of gut bacteria from 3 to 6 months of age, but a larger shift unfolded from 6 to 12 months.

Gut bacterial diversity

As expected, growing diversification with older age was also represented by the increase of alpha diversity (age effect: Shannon $t(436) = 11.74$, $b = 0.13$ per month older, Observed $t(436) = 20.19$, $b = 13.09$, Chao1 $t(436) = 17.46$, $b = 14.49$, all $p < 0.001$; Figure 2B). Batch affected diversity measures (specifically the 4th run), yet, however, explained proportionally little variance in comparison to the effect of age (Shannon $t(436) = -4.24$, $b = -0.31$, Observed $t(436) = -3.48$, $b = -18.54$, Chao1

t(436) = -3.86, b = -23.68, all p < 0.001; effect size eta-squared 1.4% batch vs 26.6% age). An effect of breastfeeding was observed in Shannon only, such that predominantly breastfed infants had reduced gut bacterial diversity (t(436) = -2.04, b = -0.04, p = 0.04).

With Bray Curtis distance, we estimated beta diversity to quantify the dissimilarity between infants, which was significantly driven by age (Adonis, F(3,449) = 19.97, p = 0.001, R2 = 0.12). While there was a substantial overlap at 3 and 6 months, there was a slight shift of the centroid, indicating some changes to the gut bacteria composition (ellipses on Figure 2C). Beta diversity at 12 months showed a notable shift in centroid compared and increasingly less overlap with beta diversity at 3 and 6 months.

Bacterial Maturation Index

The random forest model classified infants based on gut bacterial profiles to their age with 83.9% accuracy. Of particular interest were those bacterial profiles that deviated from correct age predictions: the model misclassified 14.5% of infants at 3 months (thereof 20 infants assigned to the older age 6 months, and 2 infants to even 12 months), 25.9% of infants at 6 months (thereof 33 infants assigned to younger age 3 months, yet 9 infants to 12 months), and 7.2% of infants at 12 months (1 infant assigned to 3 months, and 10 infants assigned to 6 months). The 20 top ranking age discriminatory bacterial OTUs comprised exclusively Firmicutes, 17 from Clostridiales order (6 from the *Lachnospiraceae* family), and 2 from Lactobacillales order, and 1 from an unknown order.

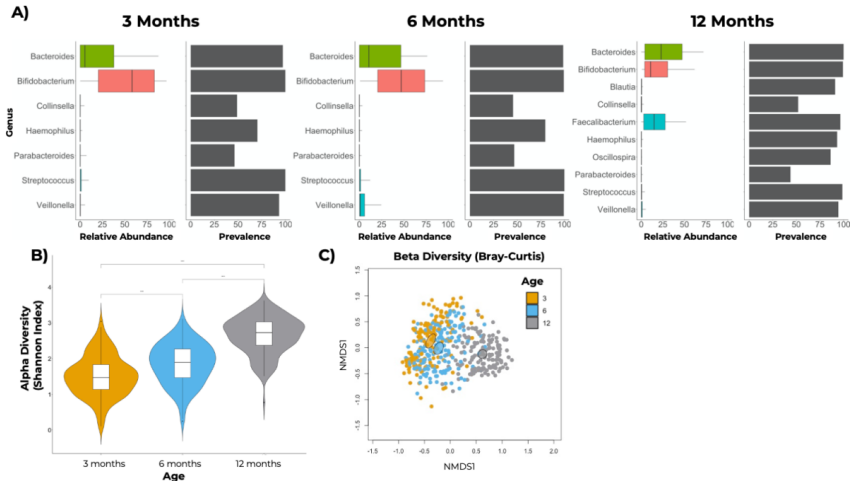

Figure 2. Maturational dynamics of infant gut bacteria across the first year of life. A) Prevalence and abundance (%) of core bacterial genera at 3, 6, and 12 months of age. B) Significant increase of Shannon index with age, with $p < 0.001$ among all alpha diversity measures: Observed, Shannon, and Chao1. C) Bray-Curtis based beta diversity to illustrate the age groups. 3 and 6 months of age highly overlap, with a close location of the centroids (ellipsis depicting 99% CI). The centroid for age 12 months is more notably distant to the younger groups, however, an overlap remains.

Enterotype

In the next step, we determined the representation of bacterial profiles as enterotypes in the sample. Weighted unifrac was performed across all ages with the clustering algorithm of Partitioning Around Medoids and identified the best fit in a 2-cluster solution of 2 distinct enterotypes (A and B) with each representative bacterial composition. The two identified enterotypes differed primarily in the abundance of *Bifidobacterium* and *Bacteroides.* While enterotype A was primarily characterized by highly abundant *Bifidobacterium* and a low abundance of *Bacteroides*, enterotype B demonstrated the reverse pattern. Enterotype B additionally maintained more core genera, including some Firmicutes (Figure 3).

The number of infants assigned to each enterotype significantly changed across age. Specifically, more infants were assigned to enterotype A at 3 and 6 months (69.8%

and 65.2%), whereas to enterotype B at 12 months of age (83.6%, $\chi^2_{(3)} = 107.46$, $p < 0.001$).

The developmental enterotype evolution patterns uncovered that approximately half of the infant population switched from enterotype A to enterotype B in the transition from 6 to 12 months (54.0 % "Switchers"). Nearly a third of infants consistently maintained enterotype B (29.0 % "Bacteroides"), and a minority of infants remained with enterotype A (12.5 % "Bifidobacterium"). A small fraction of the infant population converted from B to A (4.6% "Reverser").

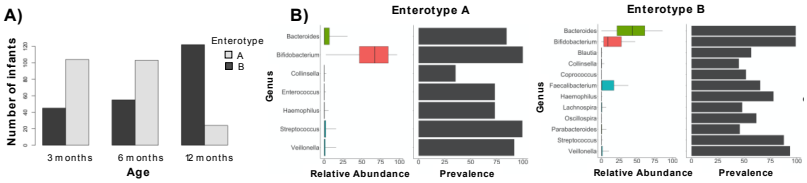

Figure 3. Weighted unifrac Partitioning Around Medoids analysis reveals two distinct enterotypes of gut bacteria composition in infancy. A) Number of infant stool samples assigned to enterotype A or B at each age based on a clustering approach. B) Characterization of the two infant enterotypes through abundance and prevalence of bacterial genera. Enterotype A is characterized by a high abundance and prevalence of *Bifidobacterium*, while enterotype B is characterized by the highest abundance of *Bacteroides*.

Investigation of the sleep-gut link

Extent of infant daytime sleep is associated with gut bacterial diversity

We then examined whether and how infant sleep patterns relate to gut bacterial diversity. We computed three multilevel models across each age, with alpha diversity as the outcome and sleep composites as independent variables (controlling for age, breastfeeding, sex, batch). We identified a negative association between *Sleep Day* and alpha diversity: infants with more diverse bacterial profiles generally demonstrated less predominant daytime sleep, thereby suggesting more “advanced”

patterns in both domains even when controlling for age (Figure 4). The effect reached significance in Observed ($t_{(240.90)}$ = -2.14, p = 0.03) and Chao1 ($t_{(332.09)}$ = -2.29, p = 0.02) and a trend in Shannon ($t_{(392.95)}$ = -1.75, p = 0.08). The other core infant sleep domains *Sleep Night, Sleep Timing, Sleep Activity,* and *Sleep Variability* were not significantly associated with gut bacterial diversity (p > 0.1, Table 1).
To examine if this association is dynamic across the first year of life, we separated linear models for each assessment timepoint. At 3 months alpha diversity was strongly linked to *Sleep Day* (Observed $t_{(83.66)}$ = -2.95, p = 0.004, Chao1 $t_{(98.66)}$ = -2.73, p = 0.008, Shannon $t_{(105.54)}$ = -2.11, p = 0.04), which was not the case at older ages (all p > 0.1).

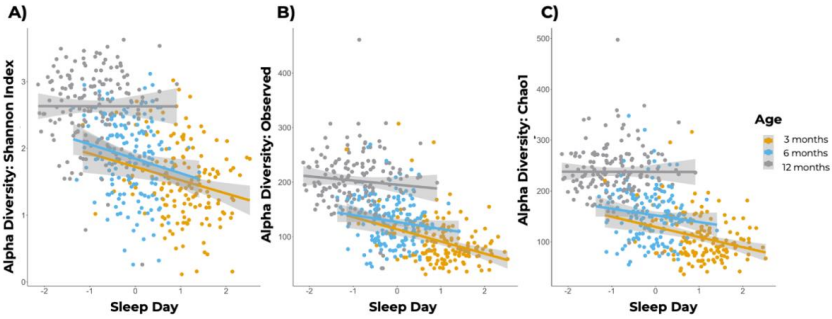

Figure 4. One representative imputation demonstrates the association between alpha diversity and *Sleep Day*, the primary infant sleep composite characterizing duration and number of daytime naps and their regularity. The finding shows that infants with more daytime sleep have lower alpha diversity. A) Shannon Diversity was significantly associated with *Sleep Day* at 3 months (p = 0.04), yet not at other ages. B) Observed diversity was significantly associated with *Sleep Day* across all ages (multilevel model, p = 0.03) and at 3 months (p = 0.004), but not at other ages. C) Chao diversity was significantly associated with *Sleep Day* across all ages (multilevel model, p = 0.02) and at 3 months (p = 0.008), but not at other ages.

Infants with more mature bacteria wake up more at nighttime

We then used the bacterial maturation index to examine whether infants classified as older/younger than their effective age according to their gut bacteria would also show a more mature/less mature sleep pattern. We computed a multilevel model with the bacterial maturation index as the outcome, all sleep composites as predictors, while controlling for exact age, sex, breastfeeding, and batch. Indeed, *Sleep Activity* was significantly linked to the bacterial maturation index ($t_{(348.91)}$ = 2.23, p = 0.03, Table 2), indicating that infants with more mature gut bacterial profiles also showed more activity and awakenings during sleep. This effect was evident only when all infant ages were combined and disappeared when age time points were considered separately (all sleep composites p > 0.05).

Enterotype

Lastly, we investigated if sleep differs between infants assigned to enterotype A vs. enterotype B. No significant group differences in sleep composites were found neither across all time points nor within assessment time points (independent variables sleep composites, controlling for exact age, sex, and breastfeeding; all p > 0.05, Table 3).

When comparing the enterotype evolution patterns ("Switchers", "Bifidobacterium", "Bacteroides" and "Reverser") infants age 12 months with the "Bifidobacterium" pattern had significantly increased *Sleep Activity* compared to infants in the "Switcher" pattern ($t_{(120.43)}$ = 2.00, p = 0.048). Additionally, infants with "Bacteroides" evolution pattern showed higher *Sleep Variability* compared to infants in the "Switcher" pattern ($t_{(130.84)}$ = 2.27, p = 0.02).

Sleep and gut bacteria as predictors for developmental behavioral outcomes

We examined whether infant sleep behavior and gut bacterial composition, 1) relate to their concurrent behavioral abilities, and 2) whether they predict later infant behavioral skills. We ran six random-intercept cross-lagged panel models, two for each behavioral outcome (*Collective Score, Gross Motor Score, Personal Social Score*), one with all sleep composites, the other with the three gut bacterial markers. We present each predictor variable (sleep behavior, gut bacterial markers) separately and only report significant findings in the text (for b ± SE see Figure 6).

Sleep Day

Analyses for concurrent behavior at 3 months revealed a negative link between *Sleep Day* and behavioral skills, including *Collective (*z = -2.08, p = 0.04*), Gross Motor (*z = -2.46, p = 0.01) and *Personal Social* scores (z = 2.00, p = 0.045). Analyses for predictive behavior revealed a negative prediction of *Gross Motor* score at 24 months by *Sleep Day* at *12 months* (z = -2.09, p = 0.04). Thus, infant daytime sleep patterns are linked to behavior - concurrently at 3 months for multiple behavioral domains and predictively for later motor skills at age 2 years.

Sleep Night

Analysis at 3 months showed that *Sleep Night* was positively associated with the concurrent *Personal Social Score* (z = 2.80, p = 0.005). At 12 months *Sleep Night* was positively associated with concurrent *Gross Motor Score* (z = 2.09, p = 0.04). Therefore, infants with more nighttime sleep revealed increased concurrent behavioral performance at 3 and 12 months.

Sleep Activity

At 3 months, *Sleep Activity* was positively linked with the concurrent *Personal Social* behavior (z = 2.00, p = 0.045), indicating that 3 months-old infants with more activity and awakenings during the night have increased developmental performance.

Sleep Timing

Sleep Timing at 6 months negatively predicted the *Personal social Score* at 12 months (z = -2.56, p = 0.008). Thus, infants with later sleep times at age 6 months had lower behavioral developmental scores at 12 months.

Sleep Variability

Analyses showed that at 6 months *Sleep Variability* was positively associated with the concurrent *Gross Motor Score* (z = 2.44, p = 0.02). Further, *Sleep Variability* at 6 months positively predicted the *Collective Score* at 12 months (z = 2.05, p = 0.04). Overall, infants with more variable sleep patterns had increased behavioral developmental scores - both concurrently and predictively.

Gut bacteria and behavioral development

Gut Bacterial Diversity

Analyses at infant age 3 months revealed that the gut bacterial diversity was positively associated with the concurrent *Gross Motor Score* ($z = 2.15$, $p = 0.03$). At 12 months, alpha diversity was positively associated with the concurrent *Collective Score* ($z = 2.00$, $p = 0.045$) and *Gross Motor Score* ($z = 2.10$, $p = 0.04$). Therefore, infants with higher alpha diversity performed better in concurrent behavioral assessments.

Bacterial Maturity Index

Analyses showed that the bacterial maturity index at infant age 3 months was positively associated with the concurrent *Gross Motor score (*$z = 2.69$, $p = 0.007$). Therefore, infants with more mature gut bacteria at 3 months scored higher in motor behavior.

Enterotype

Predictive analyses showed that the enterotype A at 6 months predicted higher *Gross Motor Scores* at 12 months ($z = 2.12$, $p = 0.03$).

	Same Age			Lagged (+ 1 assessment timepoint)		
3 Months	Collective Score	Gross Motor	Personal Social	Collective Score	Gross Motor	Personal Social
Sleep Measures						
Sleep Activity	0.15 ± 0.14	0.14 ± 0.12	0.21 ± 0.10	0.12 ± 0.10	0.14 ± 0.12	0.09 ± 0.12
Sleep Night	0.10 ± 0.16	0.12 ± 0.15	0.26 ± 0.09	-0.004 ± 0.8	0.08 ± 0.09	0.10 ± 0.08
Sleep Day	-0.10 ± 0.05	0.10 ± 0.04	-0.08 ± 0.04	-0.18 ± 0.36	0.45 ± 0.41	-0.42 ± 0.37
Sleep Timing	-0.08 ± 0.08	-0.12 ± 0.07	-0.14 ± 0.08	0.05 ± 0.11	0.23 ± 0.13	-0.12 ± 0.12
Sleep Variability	0.009 ± 0.13	-0.18 ± 0.11	0.16 ± 0.11	0.16 ± 0.09	0.01 ± 0.12	0.03 ± 0.10
Gut bacteria Measures						
Alpha diversity	0.09 ± 0.12	0.16 ± 0.08	0.11 ± 0.09	0.07 ± 0.13	0.09 ± 0.16	0.14 ± 0.13
Enterotype	0.13 ± 0.08	0.07 ± 0.06	0.06 ± 0.06	0.20 ± 0.13	0.27 ± 0.16	-0.05 ± 0.15
Bacterial Maturation Index	0.23 ± 0.16	0.31 ± 0.11	-0.07 ± 0.12	-0.04 ± 0.08	0.13 ± 0.10	-0.06 ± 0.09
6 Months						
Sleep Measures						
Sleep Activity	-0.03 ± 0.13	0.14 ± 0.12	-0.11 ± 0.11	0.10 ± 0.10	-0.02 ± 0.13	0.27 ± 0.14
Sleep Night	-0.03 ± 0.13	-0.09 ± 0.11	0.04 ± 0.09	-0.05 ± 0.09	-0.08 ± 0.12	0.11 ± 0.10
Sleep Day	0.01 ± 0.04	0.01 ± 0.03	-0.06 ± 0.03	0.11 ± 0.39	-0.30 ± 0.45	0.41 ± 0.47
Sleep Timing	-0.06 ± 0.09	0.09 ± 0.08	-0.12 ± 0.08	-0.17 ± 0.10	-0.09 ± 0.12	-0.31 ± 0.12
Sleep Variability	0.10 ± 0.14	0.29 ± 0.12	0.06 ± 0.11	0.19 ± 0.09	0.11 ± 0.12	0.16 ± 0.12
Gut bacteria Measures						
Alpha diversity	-0.17 ± 0.20	0.07 ± 0.07	0.07 ± 0.08	0.11 ± 0.13	0.15 ± 0.16	0.28 ± 0.16
Enterotype	0.09 ± 0.09	-0.02 ± .05	-0.05 ± 0.05	0.17 ± 0.14	0.33 ± 0.15	-0.08 ± 0.17
Bacterial Maturation Index	-0.50 ± 0.34	0.05 ± 0.12	0.06 ± 0.12	-0.13 ± 0.09	-0.03 ± 0.10	-0.13 ± 0.11
12 Months						
Sleep Measures						
Sleep Activity	-0.16 ± 0.14	-0.11 ± 0.12	-0.18 ± 0.11	-0.13 ± 0.12	-0.15 ± 0.12	-0.10 ± 0.14
Sleep Night	0.03 ± 0.12	0.22 ± 0.11	-0.02 ± 0.09	-0.10 ± 0.09	0.03 ± 0.09	0.0004 ± 0.09
Sleep Day	-0.03 ± 0.03	-0.008 ± 0.03	-0.03 ± 0.03	-0.42 ± 0.41	-0.98 ± 0.47	-0.78 ± 0.49
Sleep Timing	-0.05 ± 0.08	-0.006 ± 0.07	-0.06 ± 0.07	-0.09 ± 0.12	0.08 ± 0.14	-0.20 ± 0.14
Sleep Variability	-0.04 ± 0.14	-0.02 ± 0.11	-0.05 ± 0.11	-0.001 ± 0.11	0.003 ± 0.13	-0.06 ± 0.12
Gut bacteria Measures						
Alpha diversity	0.17 ± 0.08	0.16 ± 0.08	0.11 ± 0.09	0.15 ± 0.12	-0.11 ± 0.16	0.06 ± 0.14
Enterotype	-0.02 ± 0.05	-0.02 ± 0.05	-0.06 ± 0.05	-0.17 ± 0.17	-0.12 ± 0.20	-0.23 ± 0.20
Bacterial Maturation Index	0.03 ± 0.12	0.03 ± 0.11	-0.08 ± 0.13	0.05 ± 0.07	0.16 ± 0.09	-0.07 ± 0.09

Negative Effect
Positive Effect

Figure 6. Associations between infant sleep composites, the three gut bacteria measures and behavioral skills at 3, 6, and 12 months of age (β ± Standard Error). Left columns contain statistics for concurrent associations (e.g., 3 months in relation to 3 months), right columns indicate associations lagged by one assessment timepoint (e.g., 3 months in relation to 6 months). Blue color denotes positive association, while red color denotes negative associations.

DISCUSSION

We examined whether a sleep-gut link exists across the first months of human life by means of a large longitudinal cohort of healthy infants. We quantified infant sleep behavior, gut bacterial markers, and behavioral developmental status at ages 3, 6, and 12 months. Indeed, our results overall confirm the existence of a sleep-gut link in infants. Specifically, the expression of infant daytime sleep was related to alpha diversity in stool samples, and nighttime awakenings were related to the bacterial

maturity index as well as the enterotype. Further, the sleep-gut link was dynamic across the infant period, such that the specific sleep-gut associations changed across development. Finally, both - sleep behavior and gut bacteria were connected with infant behavioral developmental status, with the most prominent associations of sleep-behavior and gut-behavior at age 3 months.

Maturation of Gut Bacteria

Composition and Diversity

Gut bacteria composition and its maturation emerged in the patterns as expected for infants. We reported a marked increase in alpha diversity but a decrease in beta diversity across the first year of life, which agrees with previous findings [118,119]. As previously reported, *Bifidobacterium* was the most abundant genus in the first few months of life, while at 12 months, *Bacteroides* became increasingly abundant [120,121]. Furthermore, the observed increasing prevalence and abundance of Firmicutes at 12 months is in alignment with previous research [120].

Enterotypes

We provide new evidence that healthy infants can be assigned to two enterotypes based on their gut bacterial profiles. One enterotype (A) is characterized by high abundance of *Bifidobacterium*; the other (B) is represented by increased abundance of *Bacteroides.* In contrast, a study with neonates and young infants reported the existence of 3 enterotypes infants – one characterized by a high abundance of Proteobacteria, another by a high abundance of Actinobacteria, and a third with highly abundant Firmicutes [122]. Interpretations considered that these enterotypes differed geographically and that in Western societies, the Actinobacteria enterotype was the most common. The latter likely corresponds to enterotype A in our sample (characterized by high *Bifidobacterium*). Relevant to this discussion is our reporting that most infants switch across their first year of life from the *Bifidobacterium* enterotype (A) to the *Bacteroides* enterotype (B). The enterotypes identified in our Swiss sample differ from the ones reported in an American sample in Carlson et al., 2018 [37], which identified 3 enterotypes at 12 months old. Namely, while we similarly observe an increase in abundance of *Faecalibacterium* at 12 months, our computation does yet not identify a clearly separable third cluster. Of note, our sample possibly reveals overall reduced gut bacterial diversity, due to our strict

inclusion criteria (infants breastfed for 3 months and born naturally, i.e., no C-section), which were chosen to reduce interindividual variance of known influences, yet which may have influenced the enterotype characterization.

Sleep

Sleep Day

Our results demonstrate that infant daytime sleep (*Sleep Day*) is linked to infants' gut bacterial profile (alpha diversity): Infants who slept more during the day had lower diversity compared to infants who slept proportionally less during the day. The effect was most substantial at 3 months and gradually decreased thereafter. This finding is exciting because we recently identified *Sleep Day* also as the most relevant player linked to infant behavioral development among other comprehensively tested sleep behaviors [4]. Yet, why the sleep-gut association weakens across development is unclear. Potentially, this phenomenon identifies an early sensitive period for later functionality of sleep rhythm and gut balance. It is particularly the first year of life, that entails the most drastic changes of both daytime sleep and alpha diversity [123]. Further, daytime sleep gradually decreases across infancy into the preschool years [3]. It is thus possible that in the infant period, the build-up of sleep-profiles and gut bacterial profiles are connected, while in the later period of refinement of both profiles happens more distantly, and possible includes additional factors.

Daytime sleep reflects processes of human brain development. The observation that *Sleep Day* at 12 months predicts 24-month motor development is novel and adds further evidence to the growing concept of *Sleep Day* as core maturational marker [4,124,125]. In line with this, the amount of wakefulness at birth (i.e., inverse to our *Sleep Day)* was previously positively linked to motor development [126]. A comprehensive electrophysiological investigation of naps across early childhood further supports the concept that more daytime sleep suggest a less mature neural system, as evidenced by increased sleep pressure (slow wave activity) when nap are moved to later clock times of the day [125]. Additionally, young children who habitually nap were observed to have less mature cognitive networks, assessed as hippocampal volume, compared to children who have ceased napping [124].

In adults and rodents, increased alpha diversity has been associated with better sleep quality [39,49]. Sleep quality is traditionally measured by nighttime awakenings in older children or adults. However, we did not observe any associations between *Sleep Activity* - the composite that includes nighttime awakenings - and alpha diversity in our infant sample.

Sleep Activity

Nighttime awakenings are common in infants, especially at 3 and 6 months. At 6 months, consistently sleeping through for 8 hours is observed in less than 3% of all infants [127]. Surprisingly, our results demonstrate that *Sleep Activity* at 3 months is positively linked to personal social development. We observed a notable developmental transition in the association between *Sleep Activity* with developmental outcomes. On a descriptive level, albeit not significant, connections between *Sleep Activity* and behavioral development are positive, at 6 months associations mixed, whereas at 12 and 24 months negative. We thus propose a new model that *Sleep Activity* transitions from first being a “functional” behavior with the purpose to regularly wake up in order to being fed, to second becoming a behavior not anymore linked to survival. In the latter, *Sleep Activity* becomes “dysfunctional” and turns into an indicator for reduced sleep quality. Similarly, while toddlers seem to exhibit a linear relationship between nighttime awakenings and cognitive function, this association is not linear in infants. Interestingly, the association seems to be in a reverse u-shape in infants, so that infants in the midrange scored highest on the mental development index [128].

Potentially the maturational change in the functionality of *Sleep Activity* is related to research methodology. *Sleep Activity* in actigraphy is computed from movement, and thus quantifies a subject’s motor activity which can also occur during sleep instead of uniquely the periods of wakefulness at nighttime. While we used data smoothing to only include periods of activity that are longer than 5 minutes, potentially more extended REM periods are still captured in this composite. This is particularly the case for infants with generally high levels of activity (also during sleep) [129]. Therefore, especially in the computations at earliest ages, wakefulness during the night might be overestimated and *Sleep Activity* might contain movement activity during sleep more so than effective nocturnal wakefulness. Motor activity during

sleep in infants includes numerous twitches in REM sleep [130]. These twitches seem to trigger the neurophysiological maturation of the motor cortex, specifically by activating and thereby organizing sensorimotor networks [131,132].

We find an association between *Sleep Activity* and less mature enterotype patterns ("Bifidobacterium") at 12 months. Previously it has been reported that sleep disruptions decrease *Bifidobacterium* abundance [48]. However, it is of note that most infants assigned to enterotype B nonetheless demonstrate increased relative abundance of *Bifidobacterium* than reported in adults [119]. Likely two different mechanisms underlie the observed dynamics in *Bifidobacterium* based on sleep disruption vs. awakenings in infants.

Sleep Variability

In adults, high variability in sleep behaviors has been linked with poorer academic performance [66]. Surprisingly, *Sleep Variability* was positively associated with behavioral outcomes at 6 and 12 months. As sleep becomes less variable across the first year of life, higher variability is a more immature sleep behavior. Higher variability could potentially be related to parents' higher attunement to the baby's cues and personal needs. Potentially, the more variable schedule could allow the infant to sleep when it is most needed (e.g., after learning something new [133]) rather than at pre-specified times.

Sleep Night

We did not find any association of *Sleep Night* with the three gut bacterial markers. Previous studies in adults have reported changes in gut bacteria profiles after partial sleep deprivation [47], yet another study did not find solid effects of sleep restriction [50]. However, infant sleep duration was not experimentally altered in the current investigation, which may explain the lack of effects.

Infant nighttime sleep duration was linked to behavioral outcomes, such that longer sleep duration was associated with increased personal social scores at 3 months and higher gross motor scores at 12 months. This extends existing knowledge to the infant domain, as sleep duration in toddlers was previously linked to cognitive performance [134].

Sleep Timing

We did not find any association of *Sleep Timing* with our three gut bacterial markers. Previous studies had reported that disrupted circadian rhythms influenced the gut bacteria composition [42,44]. However, while the infants in our study showed variability in the preferred *Sleep Timing,* none showed a strong disruption of circadian rhythms, which might explain a lack of effect. Furthermore, we focused on sleep behavior (e.g., bedtimes) rather than circadian patterns (e.g., inter-day stability).

We found limited evidence for an effect of *Sleep Timing* on behavioral outcomes. Late sleep timing at 6 months negatively predicted *Personal Social* development at 12 months. Potentially, this effect was only visible at 6 months, because most infants changed to earlier *Sleep Timing* from 3 to 6 months, and subsequently retained stable timing.

Gut Bacteria Behavior Link

Alpha Diversity

Behavioral development was positively linked to alpha diversity at 3 and 12 months. A negative association between alpha diversity at 12 months and development at 24 months has been previously reported [37], which we did not observe for these ages. Potentially, the different developmental behaviors investigated (visual reception, expressive language vs gross motor and personal social development) led to this divergence in results [63,135].

Enterotype

Enterotype was generally only weakly connected to behavioral development, however, 6-month-olds with enterotype A had increased motor skills at age 12 months. As most infants switch from enterotype A to enterotype B between 6 and 12 months, this period potentially reflects a sensitive transition in bacterial profiles.

Bacterial Maturity Index

The *Bacterial Maturity Index* was only associated with early motor skills. Infants with more mature bacteria showed comparably advanced motor skills at 3 months. Immature bacterial profiles have previously been linked to malnourishment [36], but

to our knwoledge no study has investigated the link of bacterial maturity to behavioral development.

Limitations

Our study uses a correlational design which does not allow for causal conclusions. To address the concerns voiced about cross-lagged panel models regarding the separation of the within-person and between-person trait like differences we used a random intercept cross-lagged panel model [117].

Due to the longitudinal data collection across 4 years, gut bacteria were analyzed across five batches. This created differences of sequencing depth between runs, which especially influenced alpha diversity. We counteracted this effect with two actions: only including samples exceeding 50'000 reads, and accounting for analysis run with a covariate whenever possible (exceptions were the random-intercept cross-lagged panel models and the models using enterotype as a predictor).

Furthermore, reads were detected in our negative control samples (water probes). However, ZOTU2, which was the most common OTU in the negative control samples, only influenced the 3 gut bacteria markers to a negligible extent.

Our findings might not generalize to infants born through c-section or who were bottle-fed in early infancy, as we purposely included natural births and breastfed infants to account for known effects in gut bacteria [120,121].

Implications

These findings are of great clinical relevance because both, sleep and gut bacteria, are modifiable. Sleep can be modified using healthy behavioral interventions through parents, e.g., education about infant sleep rhythm maturation and specific behavioral strategies to improve sleep [20,136]. Gut bacteria can be modified by nutrition or orally ingested pre- and probiotics added to infant formula [33,137]. Even though specific recommendations cannot be made with the current state of knowledge, there is large potential to alter the sleep-gut link from either direction.

Summary

This study is the first to show the existence of the sleep-gut link in infancy. Both sleep and gut bacteria undergo rapid maturational transitions, and these findings

demonstrate dynamic connections between the two. Specifically, we found that infant daytime sleep is associated with alpha diversity and motor activity during sleep, and that nighttime waking is associated with both bacterial maturity and bacterial enterotype. Furthermore, both sleep and gut bacteria are related to behavioral development across the first year of life, with the strongest association at 3 months. Potentially, this phenomenon identifies an early sensitive period for later functionality of sleep rhythm and gut bacterial balance.

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Table 1. Associations between alpha diversity and sleep behavior in the first year of life.

	Overall		**3 Months**		**6 Months**		**12 Months**	
Shannon	**Estimate ± SEM**	**P value**	**Estimate ± SEM**	**P value**	**Estimate ± SEM**	**P value**	**Estimate ± SEM**	**P value**
(Intercept)	1.55 ± 0.15	< 0.001	2.51 ± 0.76	< 0.001	-0.08 ± 0.98	0.94	7.22 ± 2.57	0.01
Exact_Age	**0.1 ± 0.02**	**< 0.001**	-0.25 ± 0.26	0.33	**0.38 ± 0.17**	**0.03**	-0.36 ± 0.22	0.10
RunSecond	-0.09 ± 0.09	0.31	-0.15 ± 0.14	0.26	-0.06 ± 0.14	0.66	-0.19 ± 0.21	0.37
RunThird	-0.11 ± 0.09	0.22	-0.06 ± 0.13	0.65	-0.11 ± 0.15	0.47	-0.27 ± 0.22	0.22
RunFourth	-0.29 ± 0.08	**< 0.001**	**-0.31 ± 0.14**	**0.02**	**-0.26 ± 0.12**	**0.03**	**-0.47 ± 0.2**	**0.02**
RunFifth	-0.05 ± 0.11	0.66	0.49 ± 0.41	0.23	-0.27 ± 0.23	0.24	-0.12 ± 0.2	0.54
sexfemale	-0.05 ± 0.06	0.38	-0.11 ± 0.1	0.27	-0.09 ± 0.1	0.34	0.05 ± 0.1	0.63
BreastFeeding_Yes	-0.13 ± 0.09	0.12	-	-	-0.12 ± 0.18	0.53	-0.2 ± 0.11	0.07
Sleep_Activity	0.02 ± 0.03	0.66	0.06 ± 0.06	0.28	-0.01 ± 0.06	0.92	0.01 ± 0.07	0.86
Sleep_Day	-0.09 ± 0.05	0.08	**-0.2 ± 0.09**	**0.04**	-0.15 ± 0.1	0.12	0.04 ± 0.09	0.64
Sleep_Night	0 ± 0.03	0.98	-0.02 ± 0.05	0.73	-0.04 ± 0.05	0.40	0.05 ± 0.06	0.46
Sleep_Timing	0.01 ± 0.03	0.86	0.03 ± 0.05	0.58	-0.02 ± 0.06	0.78	0.04 ± 0.07	0.56
Sleep_Variability	-0.02 ± 0.03	0.62	0.01 ± 0.05	0.83	-0.1 ± 0.06	0.14	-0.03 ± 0.05	0.64
Observed overall	**Estimate ± SEM**	**P value**	**Estimate ± SEM**	**P value**	**Estimate ± SEM**	**P value**	**Estimate ± SEM**	**P value**
(Intercept)	76.6 ± 12.16	< 0.001	112.18 ± 51.91	0.03	-27.47 ± 73.19	0.71	628.97 ± 228.06	0.01
Exact_Age	**10.94 ± 1.3**	**< 0.001**	-0.49 ± 17.65	0.98	**28.16 ± 12.29**	**0.02**	-34.77 ± 19.58	0.08
RunSecond	-7.7 ± 6.65	0.25	-12.4 ± 9.81	0.21	2.06 ± 10.49	0.84	-17.78 ± 18.6	0.34
RunThird	-2.35 ± 6.7	0.73	3.95 ± 8.46	0.64	-6.89 ± 11.19	0.54	-12.27 ± 19.38	0.53

RunFourth	**-16.48 ± 6.35**	**0.01**	**-14.47 ± 9.25**	0.12	-10.77 ± 8.73	0.22	-30.01 ± 17.91	0.10
RunFifth	-2.91 ± 8.45	0.73	10.17 ± 27.08	0.71	-7.53 ± 17.01	0.66	-9.04 ± 17.66	0.61
sexfemale	-1.04 ± 4.83	0.83	1.08 ± 6.59	0.87	-0.64 ± 7.08	0.93	-0.32 ± 8.57	0.97
BreastFeeding_Yes	-4.81 ± 7.01	0.49	-	-	-3.88 ± 13.54	0.78	-7.93 ± 9.57	0.41
Sleep_Activity	1.4 ± 2.73	0.61	0.87 ± 3.85	0.82	4.75 ± 4.59	0.30	-4.13 ± 6.77	0.54
Sleep_Day	**-8.57 ± 4**	**0.03**	**-20.36 ± 6.9**	**0.004**	-4.04 ± 7.02	0.57	0.32 ± 7.81	0.97
Sleep_Night	-0.63 ± 2.33	0.79	-5.42 ± 3.24	0.10	-0.28 ± 3.72	0.94	1.26 ± 5.73	0.83
Sleep_Timing	-0.75 ± 2.63	0.78	-3.47 ± 3.6	0.34	-1.92 ± 4.05	0.64	6.61 ± 5.88	0.26
Sleep_Variability	-0.7 ± 2.51	0.78	6.34 ± 3.71	0.09	-1.66 ± 4.65	0.72	-7.85 ± 4.92	0.11
Chao1 Overall	**Estimate ± SEM**	**P value**	**Estimate ± SEM**	**P value**	**Estimate ± SEM**	**P value**	**Estimate ± SEM**	**P value**
(Intercept)	96.47 ± 13.42	< 0.001	124.28 ± 59.19	0.04	-39.76 ± 86.41	0.65	808.4 ± 251.59	< 0.001
Exact_Age	**12.36 ± 1.39**	**< 0.001**	2.25 ± 20.15	0.91	**34.89 ± 14.55**	**0.02**	**-46.64 ± 21.51**	**0.03**
RunSecond	-12.74 ± 7.57	0.09	-17.93 ± 10.96	0.10	-3.72 ± 12.72	0.77	-22.86 ± 20.36	0.26
RunThird	-5.69 ± 7.7	0.46	1.07 ± 9.77	0.91	-12.05 ± 13.51	0.37	-16.16 ± 21.4	0.45
RunFourth	**-19.27 ± 7.24**	**0.01**	-19.76 ± 10.83	0.07	-9.93 ± 10.58	0.35	-37.07 ± 19.63	0.06
RunFifth	-10.56 ± 9.85	0.28	0.55 ± 31.37	0.99	-10.4 ± 19.72	0.60	-18.73 ± 19.38	0.34
sexfemale	0.42 ± 5.45	0.94	6.5 ± 7.59	0.39	-2.18 ± 8.7	0.80	1.06 ± 9.37	0.91
BreastFeeding_Yes	-5.82 ± 8.13	0.47	-		-2.49 ± 16.4	0.88	-10.56 ± 10.68	0.32
Sleep_Activity	1.58 ± 3.12	0.61	-0.25 ± 4.43	0.96	6.64 ± 5.54	0.23	-4.59 ± 7.22	0.53
Sleep_Day	**-9.73 ± 4.25**	**0.02**	**-20.55 ± 7.53**	**0.01**	-5.31 ± 8.54	0.54	0.29 ± 8.26	0.97
Sleep_Night	-0.62 ± 2.65	0.82	-5.75 ± 3.71	0.12	0.09 ± 4.53	0.98	0.18 ± 6.2	0.98
Sleep_Timing	-1.58 ± 3	0.60	-3.98 ± 4.14	0.34	-2.22 ± 4.89	0.65	6.23 ± 6.45	0.34

Sleep_Variability	-0.87 ± 2.9	0.76	7.7 ± 4.23	0.07	-3.05 ± 5.62	0.59	-8.75 ± 5.4	0.11

Note. Standard errors of the mean are reported. Breastfeeding was not included in the model at 3 months because all infants were (over 50%) breastfed at that age. Bold shows significant associations ($P < 0.05$).

Table 2. Associations between the Bacterial Maturation Index and sleep composites across the first year of life.

	Overall		**3 months**		**6 months**		**12 months**	
	Estimate ± SEM	**P value**	**Estimate ± SEM**	**P value**	**Estimate ± SEM**	**P value**	**Estimate ± SEM**	**P value**
(Intercept)	1.88 ± 0.5	< 0.001	-3.42 ± 1.95	0.08	-6.48 ± 3.42	0.06	16.8 ± 9.31	0.07
Exact_Age	**-0.17 ± 0.06**	**< 0.001**	**1.64 ± 0.67**	**0.02**	**1.2 ± 0.57**	**0.04**	-1.47 ± 0.8	0.07
RunSecond	-0.23 ± 0.28	0.40	-0.46 ± 0.36	0.20	-0.08 ± 0.48	0.88	-0.1 ± 0.74	0.89
RunThird	0.05 ± 0.28	0.86	-0.56 ± 0.32	0.08	0.72 ± 0.52	0.17	0.11 ± 0.77	0.89
RunFourth	-0.37 ± 0.25	0.15	-0.49 ± 0.36	0.17	-0.01 ± 0.4	0.98	-0.3 ± 0.71	0.67
RunFifth	0.21 ± 0.35	0.55	-1.17 ± 1.04	0.26	1 ± 0.81	0.22	0.07 ± 0.7	0.92
sexfemale	-0.24 ± 0.19	0.19	-0.3 ± 0.25	0.24	-0.36 ± 0.33	0.28	-0.24 ± 0.35	0.51
BreastFeeding_Yes	**-0.76 ± 0.29**	**0.01**	-	-	-0.88 ± 0.64	0.17	-0.58 ± 0.39	0.14
Sleep_Activity	**0.26 ± 0.12**	**0.03**	0.2 ± 0.15	0.16	0.37 ± 0.22	0.10	-0.02 ± 0.27	0.94
Sleep_Day	-0.15 ± 0.18	0.40	-0.29 ± 0.25	0.25	0.03 ± 0.35	0.94	-0.28 ± 0.34	0.43
Sleep_Night	0.06 ± 0.1	0.53	0.16 ± 0.12	0.18	0.02 ± 0.17	0.91	-0.2 ± 0.24	0.39
Sleep_Timing	-0.07 ± 0.11	0.54	-0.02 ± 0.13	0.87	-0.24 ± 0.19	0.21	0.1 ± 0.24	0.66
Sleep_Variability	-0.04 ± 0.11	0.72	0.05 ± 0.14	0.71	-0.06 ± 0.22	0.78	-0.23 ± 0.2	0.25

Note. Standard errors of the mean are reported. Breastfeeding was not included in the model at 3 months because all infants were (over 50%) breastfed at that age. Bold marks significant associations (P < 0.05).

Table 3. Associations between infant enterotype (A or B) with sleep variables in the first year of life.

	Overall		**3 months**		**6 months**		**12 months**	
	Estimate ± SEM	**P value**	**Estimate ± SEM**	**P value**	**Estimate ± SEM**	**P value**	**Estimate ± SEM**	**P value**
(Intercept)	1.71 ± 0.71	0.02	-2.73 ± 3.01	0.37	7.38 ± 4.14	0.08	-26.38 ± 12.2	0.03
Exact_Age	**-0.3 ± 0.08**	**< 0.001**	1.26 ± 1.04	0.23	-1.26 ± 0.7	0.08	**2.11 ± 1.03**	**0.04**
RunSecond	-0.28 ± 0.43	0.51	-	-	0.01 ± 0.56	0.99	-	-
RunThird	0.46 ± 0.44	0.29	-	-	0.44 ± 0.64	0.49	-	-
RunFourth	0.51 ± 0.4	0.20	-	-	-0.49 ± 0.47	0.30	-	-
RunFifth	-0.88 ± 0.54	0.11	-	-	-1.06 ± 0.85	0.22	-	-
sexfemale	0.13 ± 0.29	0.64	0.36 ± 0.38	0.34	0.32 ± 0.38	0.40	-0.25 ± 0.49	0.61
BreastFeeding_Yes	0.46 ± 0.39	0.24	-	-	0.63 ± 0.69	0.37	0.13 ± 0.54	0.82
Sleep_Activity	0.05 ± 0.17	0.76	-0.05 ± 0.21	0.83	-0.15 ± 0.25	0.54	0.7 ± 0.37	0.06
Sleep_Day	-0.15 ± 0.24	0.53	-0.1 ± 0.35	0.77	0.44 ± 0.39	0.26	-0.41 ± 0.43	0.35
Sleep_Night	-0.11 ± 0.15	0.45	-0.08 ± 0.18	0.65	0 ± 0.21	1.00	-0.19 ± 0.32	0.56
Sleep_Timing	-0.17 ± 0.17	0.31	-0.26 ± 0.21	0.22	-0.31 ± 0.22	0.18	-0.07 ± 0.34	0.85
Sleep_Variability	0.04 ± 0.16	0.80	0.23 ± 0.21	0.28	0.2 ± 0.25	0.43	0.01 ± 0.31	0.96

Note. Standard errors of the mean are reported. Breastfeeding was not included in the model at 3 months because all infants were (over 50%) breastfed at that age. Run could not be included for models at 3 and 12 months because not all combinations of Run/Enterotype existed at these timepoints. Positive estimates signify associations with enterotype A. Enterotype A is linked with higher abundance of *Bifidobacterium* while enterotype B is linked with higher abundance of *Bacteroides.* Bold marks significant associations ($P < 0.05$).

6.5 ARTICLE 5

Across-night dynamics in traveling sleep slow waves throughout childhood

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Abstract

Study Objectives: Sleep slow waves behave like traveling waves and are thus a marker for brain connectivity. Across a night of sleep in adults, wave propagation is scaled down, becoming more local. Yet, it is unknown whether slow wave propagation undergoes similar across-night dynamics in childhood—a period of extensive cortical rewiring. Methods :High-density electroencephalography (EEG; 128 channels) was recorded during sleep in three groups of healthy children: 2.0–4.9 years (n = 11), 5.0–8.9 years (n = 9) and 9.0–16.9 years (n = 9). Slow wave propagation speed, distance, and cortical involvement were quantified. To characterize across-night dynamics, the 20% most pronounced (highest amplitude) slow waves were subdivided into five time-based quintiles. Results: We found indications that slow wave propagation distance decreased across a night of sleep. We observed an interesting interaction of across-night slow wave propagation dynamics with age ($p < 0.05$). When comparing the first and last quintiles, there was a trend level difference between age groups: 2- to 4.9-year-old children showed an 11.9% across-night decrease in slow wave propagation distance, which was not observed in the older two age groups. Regardless of age, cortical involvement decreased by 10.4%–23.7% across a night of sleep. No across-night changes were observed in slow wave speed. Conclusions: Findings provide evidence that signatures of brain connectivity undergo across-night dynamics specific to maturational periods. These results suggest that across-night dynamics in slow wave propagation distance reflect heightened plasticity in underlying cerebral networks specific to developmental periods.

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Across-night dynamics in traveling sleep slow waves throughout childhood

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ABSTRACT

Study Objectives: Sleep slow waves behave like traveling waves and are thus a marker for brain connectivity. Across a night of sleep in adults, wave propagation is scaled down, becoming more local. Yet, it is unknown whether slow wave propagation undergoes similar across-night dynamics in childhood – a period of extensive cortical rewiring.

Methods: High-density EEG (128 channels) was recorded during sleep in three groups of healthy children: 2.0 – 4.9 y (n=11), 5.0 – 8.9 y (n=9) and 9.0 – 16.9 y (n=9). Slow wave propagation speed, distance and cortical involvement were quantified. To characterize across-night dynamics, the 20% most pronounced (highest amplitude) slow waves were subdivided into five time-based quintiles.

Results: We found indications that slow wave propagation distance decreased across a night of sleep. We observed an interesting interaction of across-night slow wave propagation dynamics with age ($p < 0.05$). When comparing the first and last quintiles, there was a trend level difference between age groups: 2 – 4.9 year old children showed an 11.9% across-night decrease in slow wave propagation distance, which was not observed in the older two age groups. Regardless of age, cortical involvement decreased by 10.4%-23.7% across a night of sleep. No across-night changes were observed in slow wave speed.

Conclusions: Findings provide evidence that signatures of brain connectivity undergo across-night dynamics specific to maturational periods. These results suggest that across-night dynamics in slow wave propagation distance reflect heightened plasticity in underlying cerebral networks specific to developmental periods.

Keywords: brain connectivity, function of sleep, high-density EEG, neurodevelopmental marker, sleep regulation

Statement of significance
Sleep is the most ideal setting for measuring maturational changes in brain activity and connectivity without influences of motivation and attention. Sleep slow waves propagate across the cortex with traveling patterns relating to age and intra-hemispheric brain myelin content. Here we examine the across-night dynamics of slow wave propagation in 29 healthy children and adolescents (2 – 16 years). Our results show an across-night decrease in slow wave distance most apparent in preschool-age children. The current findings indicate age-related differences in across-night dynamics of traveling wave propagation and propose specific electrophysiological brain markers for detecting heightened developmental plasticity.

INTRODUCTION

Sleep is an ideal state for measuring brain activity, connectivity and the development of the cerebral cortex because motivational, attentional and contextual influences are marginal. This is especially important during development when motivational and regulatory control systems undergo divergent trajectories. [1, 2] Brain markers assessed during sleep thus reliably reflect not only the static patterns of neural activation and connectivity but also the neuronal dynamics that occur across the course of sleeping or waking. [3]

Sleep slow wave activity (0.5 – 4.5 Hz) is the most typical electrophysiological phenomenon of deep sleep. Slow wave activity is also a well-established marker of sleep need, as it is highest at sleep onset and decreases across the night. [4] The synaptic homeostasis hypothesis proposes that slow wave activity reflects synaptic strength and actively drives synaptic renormalization across the course of sleep. [5] Slow waves behave like traveling waves and their propagation dynamics can be simplified using the parameters traveling distance, speed and cortical involvement. [6] Analogous to slow wave activity, the markers of slow wave propagation also experience an across-night reduction. For example, the cortical involvement of an ordinary slow wave shrinks from the evening to the morning hours by 5% in adults, [7] reflecting decreased synchronization among neuronal units. [3] It is noteworthy that the magnitude of across-night changes is generally more pronounced in developing individuals compared to adults, as evidenced in animal[8, 9] and human data. [10-13] Yet, the magnitude of these dynamics, and whether their manifestation relates to specific developmental periods, remains unknown. To our knowledge this article is the first to report across-night dynamics in travelling patterns of sleep slow waves in children, extending previous insights into the development of inter- and intra-hemispheric sleep electroencephalographic (EEG) connectivity, [12, 14] slow wave morphology[13, 15] and the link between regional sleep and behavioural development. [11, 16]
Sleep undergoes numerous transitions throughout development that also involve changes in sleep depth[17, 18] and in EEG connectivity. [12, 14] Slow wave activity reaches a maximum during childhood and declines throughout adolescence [17]. Slow wave activity also undergoes topographical changes that parallel the maturational trajectory of cortical thickness. [19] Furthermore, slow wave morphology is transformed across development. For example, prepubertal children exhibit a steeper slope of slow waves compared to adolescents, suggesting increased synaptic strength. [20, 21] Previous studies reported an increase in EEG long-range connectivity (coherence calculated from bipolar EEG channels) across preschool-age[12] or adolescence[14]. Coherence also exhibits across-night dynamics, including a decrease in intra-hemispheric connectivity and a increase in inter-hemispheric connectivity, as investigated at preschool age. [12]

Slow wave propagation transforms throughout maturation, such that traveling distance increases by 0.2 cm per year across childhood[6] (based on a subset of data presented here). It is further known that slow wave propagation parameters are linked to intra-hemispheric white matter microstructure. [6] Accordingly, slow wave propagation patterns are a promising proxy for not only developmental but also diurnal processes of brain connectivity. Yet, it is unknown whether i) slow wave propagation undergoes across-night dynamics in childhood and adolescence, and whether ii) these changes differ with age showing maturational trajectories. Consequently, the identification of sleep-related cerebral markers may not only identify cornerstones in maturational brain modifications, but also promote insight into the role of sleep for cognitive development. Here, we quantified high-density sleep EEG (hdEEG) in children and adolescents and examined the propagation of slow waves and their dynamics across the night.

MATERIALS AND METHODS

Participants

Twenty-nine healthy children (12 females) between 2 and 16 years participated in the study. Participants were recruited via newspapers, website advertising, flyers and personal contact at community events. Before participation, children were screened via telephone interview and questionnaires for health problems and current medication use as well as personal and family history of sleep disorders, psychosis, bipolar disorder, narcolepsy, physical and developmental disabilities, head injury and chronic diseases. All participants included in the study were in excellent health, had no history of these disorders or were currently using medications. Furthermore, subjects were excluded for travel beyond two time zones within 2 months of the study, caffeine use, daily/ nightly co-sleeping, pre-term or post-term delivery or low birth weight. Sleep was stabilized according to habitual bed and wake times for five or more days before assessments (compliance was confirmed with actigraphy and parent-reported sleep diaries). Ethics approval was obtained from local Institutional Review Boards (Brown University, the University of Colorado Boulder, the University of Zurich), and study procedures were consistent with the declaration of Helsinki. Written parental consent and child assent (when appropriate) were obtained after explanation of the study.

EEG recording

Results on a subset of these participants were published previously. [6] Sleep assessments were performed at families' homes in 14 subjects (2.0 - 5.7 years, 8 males) in the Providence, RI area (USA) and in 9 subjects (5.5 - 11.3 years, 6 males) in the Boulder, CO area (USA). Six subjects (11.0 - 16.4 years, 3 males) were recorded in the sleep laboratory of the University Children's Hospital Zurich (Zurich, Switzerland). Sleep assessment times were scheduled to individual bedtimes. Subjects were awakened in the morning to allow for school participation or other obligations. The procedures were consistent across sites. Sleep quality did not differ across geographic sites (sleep efficiency: one-way ANOVA $p = 0.47$; sleep latency: one-way ANOVA $p = 0.22$). High-density (hd) EEG (128 channels; Electrical Geodesics Inc., EGI, Eugene, OR) was used for monitoring one night of sleep scheduled to habitual bedtimes. Signals were obtained with a sampling frequency of 500 Hz and referenced to the vertex for direct visualization (NetStation, version 4.5.1). Impedances were below 50 kΩ.
For sleep scoring, the signal was band-pass filtered (0.5 - 50 Hz), down-sampled to 128 Hz and poor quality channels were excluded. Artifacts were semi-automatically rejected for 20 s segments (as described in[22]). Sleep stages were visually scored for 20 s epochs according to the AASM Manual for the Scoring of Sleep and Associated Events. [23] Sleep quality was good with a relatively short sleep latency (24.4 ± 16.9 min) and a high sleep efficiency (88.8 ± 4.7 %).

Slow wave detection

EEG was pre-processed offline using NetStation (version 4.5.1) and MATLAB (Mathworks, Natick, MA, USA, version R2012a). Data was filtered using a 0.5 – 40 Hz bandpass filter, rejection of channels with artifacts and re-referencing to the mastoids. The algorithm published in Siclari et al. (2014) was used for slow wave detection and adapted for use in children and with 128 channels. At each time point the third most negative sample (2.5 % of all channels) was used to create a single negative reference envelope for detecting local and global slow waves. Specific detection criteria were applied in order to target stereotyped high-amplitude waves in this pediatric sample. Relatedly, to account for maturational effects due to slow wave morphology [amplitude, slope[20, 24]], we included only the top 20% of waves (i.e., with largest amplitudes) in each subject for analysis. We determined the timing of any local maxima that occurred within ± 200 ms of the reference peak, had an amplitude of at least 25% of the peak and was within 10 ms of at least one other detected channel peak; we then chose the maxima that occurred most closely to the voltage peak in each electrode. Streamlines of the slow wave propagation were calculated with a three-dimensional gradient (2 for direction, 1 for timing). Consistent with our previous work in children, [6] we focused on slow wave parameter speed, distance and cortical involvement (Figure 1). Slow wave distance was calculated as the length on the scalp of the longest streamline in cm. Slow wave speed incorporated scalp distance in cm and the longest streamline time delay (i.e., estimated distance divided by time). Cortical involvement quantified the percentage of electrodes in which the slow wave was detected relative to the total number of electrodes.

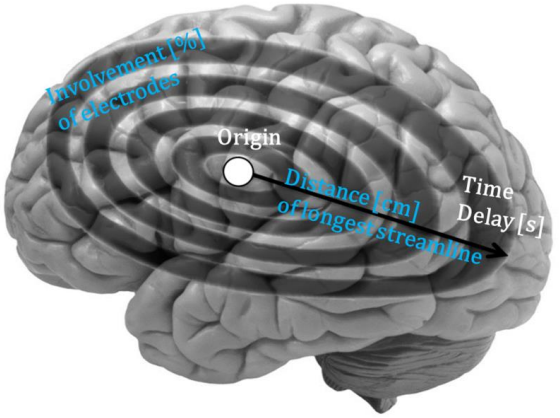

Figure 1. Slow wave propagation metrics**.** Slow wave propagation was quantified with three key metrics: slow wave distance, slow wave speed and cortical involvement.

Wave quintiles were calculated for a quantification of across-night dynamics. This approach was utilized in each subject in order to account for individual differences in numbers of waves. The number of detected waves was identified, and slow waves were assigned to the first (1-20%), second (21-40%), third (41-60%), fourth (61-80%) or fifth (81-100%) quintile. Per quintile, we calculated the median of all included slow waves. The median was used because the propagation metrics can occur with skewed distribution. [6]

Statistical Analysis

Analysis was performed with MATLAB (Mathworks, Natick, MA, USA, version 2012a) and R (Version 3.2, R Development Core Team, Vienna, Austria 2016). [25] Main results were analyzed using linear mixed-effects models to assess the dynamics across night, the effect of age and their interaction. Age was included with 0 representing the youngest participant's age (2.04 y). Mixed-effect models were selected because the main outcome variables (cortical involvement, slow wave distance and slow wave speed) were non-independent within participant (intraclass correlation coefficient [ICC] = 0.73, $F_{(1, 143)} = 6.75$, $p = 0.01$ for cortical involvement, ICC = 0.37, $F_{(1, 143)} = 43$, $p < 0.00001$ for slow wave distance, and ICC = 0.14, $F_{(1,143)} = 12.62$, $p = 0.0005$ for slow wave speed). A latent growth model is recommended to examine overall changes. [26] A growth curve model was thus implemented using the R package *nlme*[27] and the function *lme*. Models were estimated using restricted maximum likelihood, and model comparisons were performed using maximum likelihood. Linear was the best fit for all models. With exception of the initial model, all models included head size and sex as covariates. When a MR image was available, head size was calculated as head circumference measured from the structural MR images (in 23 subjects), otherwise it was based on EEG net size (in 6 subjects). To examine across-night trajectories, we implemented random-intercept-and-slope models. We applied both a random-intercept and a random-intercept-and-slopes model and performed comparisons with log-likelihood ANOVA. Subsequent analysis included only the better fitting model. A lag-1 autocorrelation was integrated if it significantly improved the model fit otherwise it was dropped. After

establishing this basic model, age was added as predictor in a first step, and as an interaction-term in a second step. The model with the lowest Akaike information criterion (AIC) was chosen as the best fitting model. Normal distribution of the residuals of the final models was assessed visually using histograms. Pseudo R^2 was calculated using the R package *MuMIn*[28] and the function *r.squareGLMM*.

Age groups

In the analyses where a main effect of age or an interaction with age was observed, subjects were assigned to one of three age groups: 2.0 – 4.9 y (n = 11), 5.0 – 8.9 y (n = 9) or 9.0 – 16.9 y (n = 9, Table 1). This age subdivision was data-driven and reflected EEG milestones based upon the topographical maturation status of slow wave activity. [29] Specifically, slow wave activity demonstrates an inverted U-shape trajectory across the maturational period, with slow wave activity maxima attained between 5 and 9 years of age, contingent to cortical regions. [18] The age groups selected for analysis refer to the age before (2 – 4.9 y), during (5 – 8.9 y) or after (9 -16.9 y) the time point (age) when slow wave activity maxima occur. To streamline across-night dynamics, we calculated the change (decrease or increase) from the first to the last quintile of slow waves in each subject. ANOVA was used for between-group comparison of the first/last quintile change and Wilcoxon signed rank tests were performed whenever significant group differences were found. Based upon our previous examination of traveling slow waves in childhood, [6] we refined the analysis and extended it to adolescence. To examine overall group differences, mean values across the whole night were calculated for each group and ANOVA was performed to examine group differences. When differences were found Tukey's HSD post hoc tests were performed. The alpha level was set to p = 0.05.

Table 1. *Descriptive statistics (M ± SD; N) for participant age, sex and sleep characteristics by age group.*

Group	2 – 4.9 y	5 – 8.9 y	9.00 – 16.9 y	p
Age	3.3 ± 1.1	6.7 ± 1.7	12.6 ± 2.5	< 0.001*
N (female)	11 (4)	9 (3)	9 (5)	0.58
Time in Bed (min)	609.5 ± 65.6	601.6 ± 55.0	515.6 ± 64.6	0.005*
Total Sleep Time (min)	540.8 ± 66.0	532.2 ± 67.1	467.7 ± 52.5	0.04
Sleep Latency (min)	31.4 ± 19.8	19.7 ± 11.9	22.0 ± 14.4	0.24
Sleep Efficiency (%)	88.6 ± 3.8	88.4 ± 6.3	90.9 ± 3.9	0.45
Stage 1 (%)	1.1 ± 0.4	1.6 ± 1.0	5.6 ± 4.2	0.02*
Stage 2 (%)	33.9 ± 3.6	45.0 ± 6.6	50.9 ± 4.5	< 0.001*
Stage 3 (%)	30.2 ± 7.4	29.5 ± 5.6	23.1 ± 4.8	0.04
REM (%)	34.8 ± 5.9	23.9 ± 8.1	20.4 ± 3.7	< 0.001*

Note: * *Significant after False Discovery correction.*

RESULTS

Between 813 and 7281 slow waves were detected among all whole-night recordings (Figure 2). The 9 – 16.9 year old group showed significantly more of the targeted high amplitude slow waves (3765 ± 1708, M ± SD) compared to the younger age groups (1852 ± 476, 2 – 4.9 year old children, 1908 ± 601 5 – 8.9 year old children, $F_{(2,14.39)} = 5.1$, p = 0.02, Welch correction due to unequal variances). Consistent with this finding, the number of detected slow waves was positively correlated with age ($r_{(27)} = 0.63$, p = 0.0002). Findings withstand removal of the outlier in the oldest age group ($F_{(2, 13.59)} = 5.55$, p = 0.02 for ANOVA and r(26) = 0.72, p < 0.001) correlation. Because this subject was not identified as outlier in any other measure, it was included for subsequent analyses.

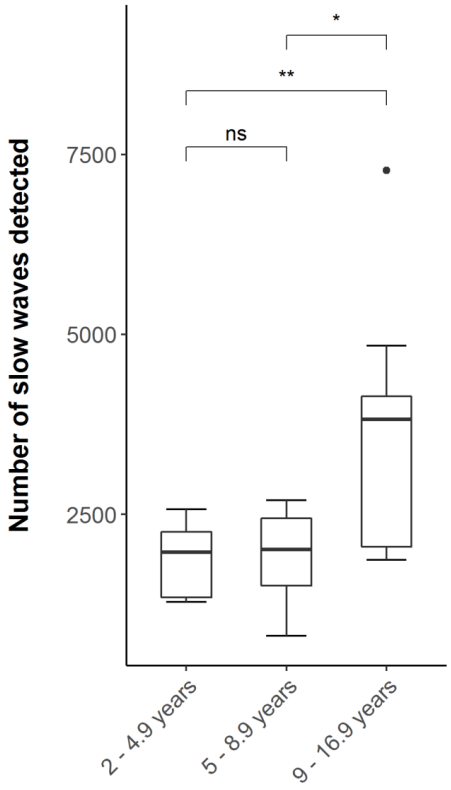

Figure 2. Number of slow waves detected in each age group: between 813 and 7281 waves were detected (813 - 4845 excluding the outlier). 9 – 16.9 year olds exhibited significantly more slow waves compared to 2 – 4.9 year olds or 5 – 8.9 year old children ($p = 0.02$). * $p < 0.05$ ** $p < 0.01$ N=29

Similar to number of detected slow waves, an age effect was found in slow wave density: 9 – 16.9 year olds showed significantly increased slow waves per minute of NREM sleep compared to 2 – 4.9 year olds or 5 – 8.9 year old children ($F_{(2,14.52)} = 5.36$, $p = 0.02$). There was no effect of sex in the number of detected slow waves ($t_{(27)} = -0.95$, $p = 0.35$) and no correlation between number of slow waves and slow wave activity (F4 $r_{(27)} = -0.03$, $p = 0.86$, C4 $r_{(27)} = -0.11$, $p = 0.58$). As expected, sleep characteristics were associated with age (Table 1[30-32]), including an age-related reduction of time spent in bed ($F_{(2,26)} = 6.58$, $p = 0.005$) and REM sleep ($F_{(2,26)} = 15.08$, $p < 0.001$), and increases in stage 1 sleep ($F_{(2,11.96)} = 5.76$, $p = 0.02$), and stage 2 sleep ($F_{(2,26)} = 30.63$, $p < 0.001$). Age-related decreases at trend-level were observed in total sleep time ($F_{(2,26)} = 3.84$, $p = 0.04$, n.s. after false discovery rate correction), and stage 3 sleep ($F_{(2,26)} = 3.79$, $p = 0.04$, n.s. after false discovery rate correction).

Slow wave traveling distance decreases across the night only in 2 - 4.9 year olds

We then investigated across-night changes in slow wave traveling distance. The random-intercept model accounting for timelag-1 autocorrelation including the predictors quintile, age; their interaction yielded the best fit when correcting for head size. Main effects reached significance, indicating a decline in slow wave distance across the night (quintile $t_{(114)} = -2.87$, $p = 0.005$, 95% CI [-0.41; -0.08]) and an age-related increase in slow wave distance, such that older participants exhibited larger

propagation distance irrespective of quintile (age $t_{(25)}$ = 2.43, p = 0.02, 95% CI [0.02; 0.25]). Additionally, a significant interaction was observed (quintile x age $t_{(114)}$ = 2.02, p = 0.046, 95% CI [0.0005; 0.05]) indicating that across-night trajectories in traveling distance change with age (Table 2). To streamline the reported age x quintile interaction, we quantified the percent change from the first to the last quintile of slow wave distance, which revealed a group difference at trend level ($F_{(2,26)}$ = 3.00, p = 0.07). Although a Wilcoxon signed rank test indicated a decrease in slow wave distance across the night in 2 – 4.9 year old children (88.1 ± 3.7%, p = 0.014), no across-night dynamics were observed in the older age groups (Figure 3A).

***Table 2**. Parameters for the model explaining slow oscillation distance.*

Fixed effects	b ± SE	p
Intercept	6.31 ± 4.15	0.13
Quintile	-0.24 ± 0.08	0.005
Age	0.13 ± 0.06	0.02
Quintile x Age	0.03 ± 0.01	0.046
Head size	0.04 ± 0.08	0.67
Gender	0.13 ± 0.36	0.72
Random effects		
Intercept	0.81	
Residual	0.85	
AIC	425.13	
Pseudo R^2 (marginal; conditional)	0.35; 0.66	

Note: *AIC = Akaike Information Criterion, SE = Standard Error*

Slow wave cortical involvement decreases across the night in all age groups
For cortical involvement, the linear random-intercept-and-slope model with quintile and age, but not their interaction, revealed the best fit. Adding timelag-1 autocorrelation did not improve the model fit. Both main effects significantly predicted cortical involvement (quintile $t_{(115)}$ = -3.54, p = 0.0006 95% CI [-1.3; -0.37], age $t_{(25)}$ = 3.03, p = 0.006 95% CI [0.13; 0.67]), indicating a decrease of cortical involvement across the night and a general maturational difference (Table 3); however, we found no maturational effect on across-night changes, indicated by a lower fit with the inclusion of the interaction term.

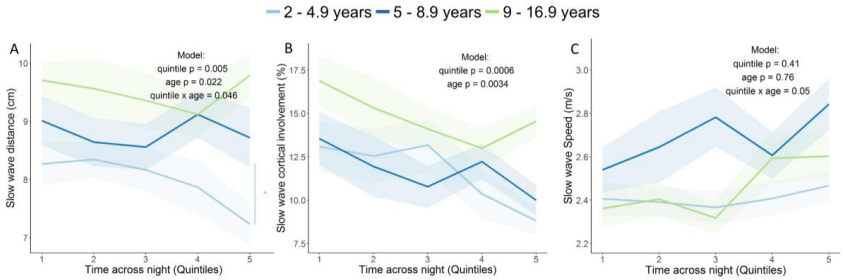

Figure 3. Across-night changes in slow wave propagation. A) Slow wave distance decreases from the first to the last quintile in 2 – 4.9 year old children (by 11.9 %) but not in 5 – 8.9 year old children and 9 – 16.9 year olds. B) Across-night decrease in cortical involvement across the night independent of age. C) Across-night dynamics in slow wave speed. There was a trend for an age x quintile interaction, however no group differences were found in changes from the first to the last quintile. The shaded area represents the SEM. * $p < 0.05$ n = 29

***Table 3**. Parameters for the model explaining slow oscillation cortical involvement.*

Fixed effects	b ± SE	p
Intercept	11.62 ± 11.13	0.3
Quintile	-0.84 ± 0.24	0.0006
Age	0.4 ± 0.13	0.006
Headsize	0.01 ± 0.21	0.95
Gender	-0.22 ± 0.97	0.82
Random effects		
Intercept	18.34	
Quintile	0.73	
Residual	8.9	
AIC	798.05	
Pseudo R^2 (marginal; conditional)	0.2; 0.6	

Note: *AIC = Akaike Information Criterion, SE = Standard Error*

First-to-last quintile percentage changes confirmed the model results by indicating no group differences in the across-night decrease of slow wave cortical involvement (76.3 ± 10.9% in the 2-4.9 year old group, 81.2 ± 10.3% in 5 – 8.9 year old children, 89.6 ± 7.2% in 9 -16.9 year old group; p = 0.63, Figure 3B). Thus, cortical involvement decreases across the night, regardless of age.

Slow wave speed exhibits no distinct maturational changes throughout the night
The final model for slow wave speed was a linear random-intercept model that included quintile, age and their interaction without autocorrelation. Main effects did not reach significance, however, the interaction between quintile and age showed a significant trend ($t_{(114)}$ = 1.97, p = 0.05, 95% CI [-0.00002; 0.01]) indicating no overall across-night change in slow wave speed, yet variation with age (Table 4). No group difference was found regarding the change in slow wave speed from the first to the last quintile (p = 0.19, Figure 3C).

Table 4. *Parameters for the model explaining slow oscillation speed.*

Fixed effects	b ± SE	p
Intercept	2.72 ± 1.18	0.02
Quintile	0.02 ± 0.02	0.41
Age	0.008 ± 0.01	0.62
Quintile x Age	0.005 ± 0.003	0.05
Headsize	- 0.008 ± 0.02	0.74
Gender	0.11 ± 0.10	0.28
Random effects		
Intercept	0.25	
Residual	0.2	
AIC	55.59	
Pseudo R^2 (marginal; conditional)	0.10; 0.65	

Note: *AIC = Akaike Information Criterion, SE = Standard Error*

Age related changes in whole night slow wave propagation
To examine the main effect of age, we then compared whole night mean data between age groups. A significant effect of age was found in slow wave distance ($F_{(2,26)}$ = 5.22, p = 0.01). Post-hoc tests revealed a difference between 2 – 4.9 year old children and 9 – 16.9 year olds (Figure 4A), confirming earlier results [6].
Mean cortical involvement differed between age groups at the trend level ($F_{(2, 26)}$ = 3.38, p = 0.05). Post-hoc tests correspondingly revealed a trend level difference between 5 – 8.9 year old children and 9 – 16.9 year olds (p = 0.07), yet not in other age groups (Figure 4B). This is again in line with previous reports on a lack of change in slow wave cortical involvement from the preschool to the school-age years.
No significant main effect of age group was found in the linear model explaining slow wave speed. Because the linear model does not account for non-linear relationships, we explored whole night differences in slow wave speed. Mean slow wave speed revealed age group differences (p = 0.049), and post-hoc tests reached trend level significance for differences between 2 – 4.9 year old and 5 – 8.9 year old children (p = 0.05, Figure 4C).

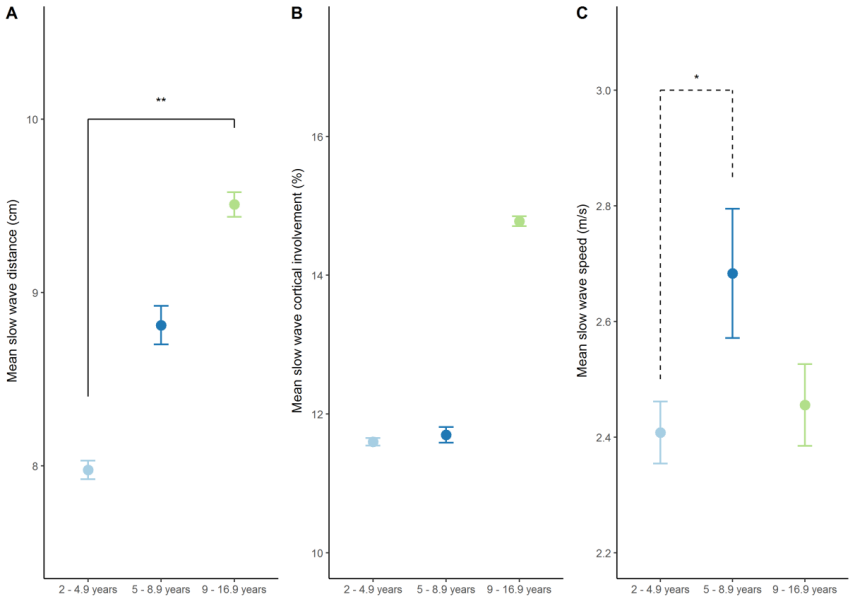

Figure 4. Slow wave whole night mean data. **A)** 9 – 16.9 year olds exhibit significantly longer slow wave distance than 2 – 4.9 year old children. **B)** No significant difference exists between age groups in slow wave cortical involvement, yet a trend is observed for increased cortical involvement in 9 – 16.9 year olds compared to 5 – 8.9 year old children. **C)** Slow wave speed differed between 2 – 4.9 year olds and 5 – 8.9 year old children at trend level. Mean ± SEM is shown. * p = 0.05 ** p < 0.01 N = 29

DISCUSSION

We investigated across-night dynamics of traveling slow waves during sleep in children and adolescents and report three main findings. First, slow wave propagation distance decreases across the night, which interacts with age. A 12% reduction from the first to last quintile was only observed in to the 2 – 4.9 year old children, and not in children older than 5 years. Additionally, there was a general maturational increase in slow wave distance (unrelated to quintile). Second, cortical involvement decreases across the night in all age groups, yet exhibits generally increased values across age. Third, slow wave speed undergoes no maturation-specific across-night dynamics. Together, these data indicate transitional periods in the across-night dynamics of sleep slow waves that are specific to certain ages. We propose that these transitions represent important cornerstones in maturational brain processes. We discuss the bidirectional implications of these findings and propose that slow waves are markers for neurodevelopment that are possibly directly involved in human brain development processes.

Using high spatial resolution EEG, we consolidated complex topographical information into EEG features. With these measures, we quantified the stability and maturation of brain connectivity. Our findings extend previous knowledge by indicating that across-night dynamics in slow wave distance are most apparent in 2 – 4.9 year old children. This observation may reflect plastic processes specific to preschool age, a period characteristic of early development with heightened plasticity and rapid learning. [33, 34] The across-night change was specifically apparent towards the morning (last quintile). Interestingly, while the oldest age group experienced a decline in preceding quintiles, the last quintile increased. The trend of change in all three wave metrics in the last percentile of the oldest group, may reflect an underlying neurophysiological process. Alternatively, decreasing stability of wave propagation measures towards the end of the sleep period is possible (even though variance does not increase). A third possible explanation is the influence of circadian effects[35] which were not controlled for in this paper. Divergence in age groups could have been influenced by maturational change in

circadian sleep regulation, linked to typically delayed sleep phase and evening chronotype in adolescents. [36] Our previous findings suggest that slow wave distance relates to white matter myelin content in the corpus callosum. [6] In an exploratory approach with a subsample of subjects, myelin water fraction was acquired with mcDESPOT magnetic resonance imaging (methods detailed in [6]). Results suggest a positive correlation between myelin water fraction in the corpus callosum with across-night changes in slow wave distance (first-to-last quintile) in 2 – 4.9 year old children ($r_{(26)}$ = 0.72, p = 0.03, corrected for age). In other words, children with reduced callosal myelin show a larger decrease in slow wave distance over the course of the night. It remains speculative whether the decrease in slow wave distance is a development-specific function of sleep as a process of active rewiring of the developing brain.

We observed an age-related effect (i.e., group difference) in slow wave traveling distance indicating larger propagation distance in older subjects, irrespective of across-night dynamics. Interestingly, this parameter also decreased across-night within the youngest age group. How can this phenomenon be explained? It is widely accepted that slow wave activity is linked to neuronal (synaptic) connectivity [5]. In developing rats, neuronal connectivity increases across a 24-hour-period despite opposite trends during the inherent period of sleep. [8] This finding indicates that the connectivity increase during the wake period generally outsizes its decrease during sleep. This imbalance within a 24-hour window is associated with a net connectivity increase – a phenomenon restricted to the maturational period. Whether similar mechanisms hold true in humans: despite a reduction in slow wave propagation distance across the period of sleep (reflecting decreasing connectivity), neuronal connectivity may nonetheless increase in waking - and in case of a positive imbalance within a 24-hour window – may also increase across the 24-h day in young children. It remains to be tested whether this net increase in connectivity may ultimately add up in anatomical connectivity change resulting in callosal myelin growth across the developmental period. [37, 38] Our novel data indicate that cortical involvement decreased across the night in all age groups, such that slow waves were locally more restricted in the morning compared to evening hours. This finding aligns with adult data which also show more local slow waves in the last hours compared to the first hours of sleep. [7] We conclude from this similarity that cortical involvement dynamics are developmentally stable. Furthermore, because propagation patterns of slow waves survive thalamectomy, [39] it was proposed that cortical involvement primarily underlies cortical synaptic connectivity. [40] The observed universal across-night decrease in cortical involvement supports the hypothesis that slow waves are tied to a reduction in neuronal connectivity at the synaptic level. [41, 42]

Mean cortical involvement generally increased with older age, which conflicts with data showing that cortical grey matter volume or thickness has been shown to increase during development, reaching a maximum in adolescence and decrease thereafter. [43] However, three aspects need to be considered: First, the timing of maximal grey matter thickness is not uniformly synchronized across the cortex, with the occipital lobe showing an increase until 20 years of age. [44] Second, our oldest age group (ages 9 – 16 years) included preadolescents and adolescents some of whom are likely to experience continued increases in grey matter volume particularly in occipital and temporal areas. [43] And third, a recent model demonstrates that synaptic refinement and reorganization can account for developmental changes in adolescence, without the requirement of synaptic pruning. [45] It is thus possible that increased connectivity at later developmental periods reflects synaptic optimization rather than pure increase or growth of synapse numbers.

Generally, our findings demonstrate that slow wave speed undergoes no pronounced change across a night and age. Given the small beta values of the main effects as well as the interaction, the dynamics of speed across the night should be considered minor. A focus of future research may extend the applied calculation of slow wave speed to more specified measures of finer resolution (considering more than one propagation streamline, inclusion of propagation direction [46]).

Our results are based on a cross-sectional based on three cohorts. Longitudinal studies are needed to corroborate these findings. Although no effects in sleep quality were detected between study sites, we cannot rule out the possibility that the inclusion of three cohorts may have affected the results. Due to only minor overlap of ages between the cohorts, controlling for site in the analyses would have masked any age effects.

A caveat of this study is that we targeted stereotyped high-amplitude slow waves by including the 20% of waves with largest amplitudes. Our approach was chosen to assess stereotyped slow waves in particular without the a priori restriction of scalp regions to minimize the potential maturational effects in EEG amplitude and frequency, [24] while at the same time allowing for topographical variation within

the sample. [20] Future studies may incorporate 1) different scalp locations, 2) unrestricted amplitude, and 3) wider ranges of speed and distance (primary and secondary propagation direction).
Our models include firstly age as linear variable and secondly age as grouping variable. The division into age groups was based on slow wave activity topographical/regional maturational state as well as the maturational state of absolute slow wave activity. This combined integration is critical for the current analyses, which extends existing approaches. [35, 47] The 9 – 16.9 year old group includes participants in preadolescence/ adolescence, a time that includes a reduction of slow wave activity. [48] This maturational decrease may have interplayed with the generation of variability in the number of detected slow waves in this age group. Of note, one may expect a similar effect creating variability in the youngest group, where slow wave activity increases due to age. [24, 29] However, the slow wave activity increase at preschool-age occurs in a narrower time window (~ 7-8 years) compared to the decrease during adolescence (~ 12 - 14 years). [24] Further, the standardized detection of waves and calculation of wave propagation metrics minimized the potential influence due to variability in sleep architecture and sleep length. In line with this is the stability of the relationship between age and number of waves detected. This relationship remains when controlling for total sleep time ($r_{(26)} = 0.57$, $p = 0.001$). Similarly, age effects in wave propagation distance ($r_{(26)} = 0.5$, $p = 0.007$) and cortical involvement ($r_{(26)} = 0.44$, $p = 0.02$) remain stable when controlled for total sleep time.
Future work is needed to separate age-effects in propagation metrics from preadolescence to adolescence by considering narrower defined age groups, which should reduce variability within groups. This approach may also yield insights concerning the surprisingly large number of waves detected in one participant of the oldest age group.

In order to capture across-night dynamics from the beginning to the end of the sleep period in a simplified manner, post-hoc analysis focused on changes from the first to the last quintile of the night. We do not exclude the possibility that additional group differences in wave propagation could be detected with percentile-by-percentile post-hoc comparisons in a sufficiently powered sample. No sex differences were observed in our models, however other studies reported such differences in developmental trajectories. [49, 50] It is possible that a larger sample could reveal such differences. Lastly, we used linear models, which neglect non-linear associations such as U-shaped relationships. However, quadratic or cubic models did not yield better fits.

In summary, our data support that traveling patterns of sleep slow waves quantify across-night dynamics in neuronal connectivity in childhood and adolescence. The age-specific observations identify specific transitional maturational patterns, which may be associated with heightened, i.e. developmental plasticity. It remains speculative whether these age-specific features are related to a neurodevelopmental function of sleep that may differ between children and adults.

Abbreviations list
ANOVA = Analysis of variance
EEG = electroencephalography
NREM = non-rapid eye movement
REM = rapid eye movement

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6.6 ARTICLE 6

Spatio-temporal properties of sleep slow waves and implications for development

Igor Timofeev, Sarah F. Schoch, Monique K. LeBourgeois, Reto Huber, Brady A. Riedner & Salome Kurth

Abstract

Objective sleep quality can be measured by electroencephalography (EEG), a non-invasive technique to quantify electrical activity generated by the brain. With EEG, sleep depth is measured by appearance and an increase in slow wave activity (scalp-SWA). EEG slow waves (scalp-SW) are the manifestation of underlying synchronous membrane potential transitions between silent (DOWN) and active (UP) states. This bistable periodic rhythm is defined as slow oscillation (SO). During its 'silent state' cortical neurons are hyperpolarized and appear inactive, while during its 'active state' cortical neurons are depolarized, fire spikes and exhibit continuous synaptic activity, excitatory and inhibitory. In adults, data from high-density EEG revealed that scalp-SW propagate across the cortical mantle in complex patterns. However, scalp-SW propagation undergoes modifications across development. We present novel data from children, indicating that scalp-SW originate centro-parietally, and emerge more frontally by adolescence. In accordance with the concept that SO and SW could actively modify neuronal connectivity, we discuss whether they fulfill a key purpose in brain development by actively conveying modifications of the maturing brain.

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Spatio-temporal properties of sleep slow waves and implications for development

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Objective sleep quality can be measured by electroencephalography (EEG), a non-invasive technique to quantify electrical activity generated by the brain. With EEG, sleep depth is measured by appearance and an increase in slow wave activity (scalp-SWA). EEG slow waves (scalp-SW) are the manifestation of underlying synchronous membrane potential transitions between silent (DOWN) and active (UP) states. This bistable periodic rhythm is defined as slow oscillation (SO). During its 'silent state' cortical neurons are hyperpolarized and appear inactive, while during its 'active state' cortical neurons are depolarized, fire spikes and exhibit continuous synaptic activity, excitatory and inhibitory. In adults, data from high-density EEG revealed that scalp-SW propagate across the cortical mantle in complex patterns. However, scalp-SW propagation undergoes modifications across development. We present novel data from children, indicating that scalp-SW originate centro-parietally, and emerge more frontally by adolescence. In accordance with the concept that SO and SW could actively modify neuronal connectivity, we discuss whether they fulfill a key purpose in brain development by actively conveying modifications of the maturing brain.

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Slow oscillations (SO) during sleep and their neuronal basis

Electroencephalography (EEG) is a non-invasive technique to measure the electrical activity of the brain. In sleep research, EEG assessments can reliably determine the objective quality and depth of sleep. When sleep deepens, the primary characteristics in the scalp-EEG are slow waves (scalp-SW) in the delta frequency (0.5–4.5 Hz) band, often quantified as slow wave activity (SWA in μV^2) [4] (see Box 1). SW and spindles dominate the EEG activity during Non-Rapid Eye Movement sleep (NREM) and particularly slow-wave sleep. In contrast, Rapid Eye Movement (REM) sleep and waking are dominated by low-amplitude high frequency activity [5,6].

How neuronal dynamics generate scalp-SW remains a core target for ultimately unraveling the dynamics of sleep. More than two decades ago, the simultaneous assessment of local field potentials (LFP, i.e. assessing extracellular electric potential) with intracellular recordings was investigated in anesthetized cats [7]. A rhythm of periodic bistability was discovered and named slow oscillation (SO, <1 Hz) [2]. These experiments demonstrated the hyperpolarization (DOWN states/silent states) of cortical neurons during depth-positive/surface negative components of LFP. In contrast, during the opposite components of LFP (depth-negative/surface positive), cortical neurons were depolarized, revealed rich synaptic activities and fired spikes [7] (UP states/active states). An identical relationship between LFP and intracellular activities was observed during natural slow wave sleep [8–10]; however, during wake and REM sleep, cortical neurons are in persistent active states showing continuous excitatory and inhibitory synaptic activity (Figure 2) [8,11–13]. Beside difference in frequency, other changes in neuronal activities recorded during SO or delta oscillations are currently unknown.

Origin and regulation of SO

The SO can emerge in isolated neocortical slabs [14,15], neocortical slices [16,17] and cortical cell cultures [18,19], overall signifying that the SO originates in the neocortex. However, functional disconnection of cortex from the thalamus temporarily disrupts cortical-SWA [15,20], and the SO rhythm is likewise absent in the thalamus of decorticated animals [21]. Further it is interesting that activity in higher-order thalamic nuclei precedes cortical active state onset [22•,23]. Together these observations indicate that cortical-SWA is controlled by subcortical structures.

Abbreviation
EEG: Electroencephalography
LFP: Local field potential
NREM: Non-rapid eye movement
REM: Rapid eye movement
SO: Slow oscillation(s)
SW: Slow wave(s)

Box 1 Slow waves, Slow Oscillations, Delta Waves, Slow Wave Activity

There is growing inconsistency regarding the terminology of brain activity during deep sleep. Recently, terms have been used interchangeably, which was possibly driven by the growing diversity of assessment methodology, analytical approach and species investigated.

Originally, the following terms were introduced:
- **Slow Oscillation (SO)** (not oscillations) **or Slow Rhythm:** cycles of cellular activity <1 Hz as a periodic process (Hz), consisting of an alternation of active and silent states, as measured with intracellular, depth electrodes or intracranial EEG from sleeping cats and humans [2].
- **Slow Wave (SW):** individual negative-positive wave in the scalp EEG or local field potential (LFP) deflection lasting several hundred milliseconds. (Incongruously, in human literature individual slow waves composing slow oscillation were called slow oscillations). Repeated slow waves as measured with intracellular or intracranial EEG are basis of slow oscillation.
- **Delta Waves or Slow Wave Activity (SWA):** wave activity in scalp EEG or intracranial EEG (μV^2), in the delta frequency 0.5–4.5 Hz, or subsets within this frequency. Sleep EEG power density is often quantified from spectral analysis (fast Fourier transform) in this frequency range in the human scalp EEG

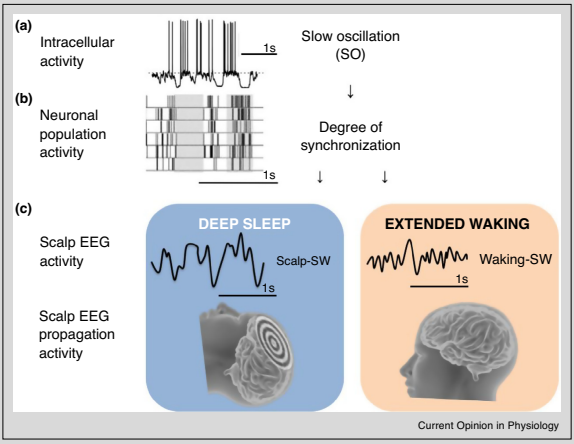
These definitions have recently been challenged due to the evolution of our understanding the following three fundaments:

1) SW in the scalp EEG reflects SO cellular activity. Specifically, the degree of synchronization among a multitude of cells determines the morphology of the scalp-SW. For example, highly synchronized SO activity among neuron populations is related to scalp-SW with high amplitude and steep slope. With low SO synchronization among neuron populations, scalp-SW demonstrate lower amplitude and flatter slope.

2) SW were discovered to travel across the cortical mantle. Integration of the first point suggests that high synchronization of SO among neuron populations leads to near-simultaneous occurrence of scalp-SW across the cortex and also broader propagation of scalp-SW. Lower local synchronization is reflected in reduced amplitudes of SWs. In contrast, low long-distance synchronization is reflected in reduced simultaneous occurrence and shorter propagation distances (local, but not global SWs). Further, the simultaneous start of SWs from different scalp locations may appear as overlapping SWs with multiple peaks.

3) Recent data from sleep deprivation experiments provide key knowledge about the local occurrence of low-frequency EEG activity (SWs, theta waves) in the waking state. These waves arise occasionally and appear as individual waves. They cannot be called SO, but they are rather SWs (Figure 1).

Figure 1



Schematic summary of the different levels of brain activity of sleep (**(a)** modified from Ref. [1], **(b)** modified from [3], **(c)** unpublished).

We propose nomenclature as outlined in the following table. We also propose for future studies to specify assessment method and/or behavioral state, i.e., scalp-SW, intracellular-SO, extracellular-SW, waking-SW, REM-SWA, etc.

Proposed term	Assessment method	Morphology / physiology	Frequency	Unit
Slow oscillation (SO)	Intracellular, extracellular unit firing, cortical LFP and cortical EEG	De- and hyperpolarized membrane potential states, cycles of spiking activity, positive and negative field deflections. Each state lasts more than 100 ms (typically above 200 ms).	<1 Hz	Hz
Slow wave (SW)	Intracellular, extracellular unit firing, cortical LFP and scalp EEG	Same as above, but individual wave, number / density / morphology thereof		Single wave
Slow wave activity (SWA)	Intracranial EEG, scalp EEG	Integrated signal, representing multiple overlapping SW	0.5–4.5 Hz	μV^2

Typical duration of the silent states of the SO in cats [24] and mice [25] is 100–200 ms. The onset of these silent states in a subset of neurons is mediated by active inhibition [26]. Thereby, a subset of fast spiking, parvalbumin positive interneurons fires prior, or at the beginning of silent states [27]. Somatostatin interneurons are also active before the onset of silent states [28,29]. The generation of small depth negative components before a large depth-positive wave [25] suggests that subsets of cortical neurons can be synchronously activated at the onset of cortical silent state.

Synchronized SO activation and termination

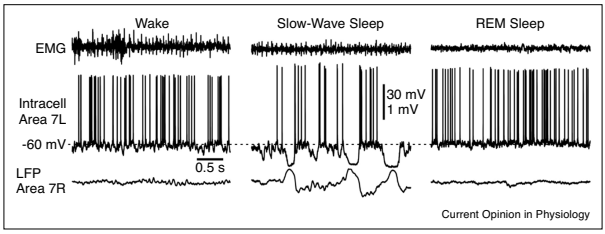
Silent states start almost simultaneously across large cortical territories [23,30] implying a central coordination of inhibitory control. One likely source of this coordination is the thalamus, as (a) thalamic inactivation disrupts synchrony of active state onsets [26], and (b) occasional firing of thalamocortical cells at the beginning of silent states directly activates parvalbumin interneurons that inhibit cortical activity [27]. Another possible source is the claustrum, which might control synchronous and widespread onset cortical silent states [31].

This inhibitory drive shifts the balance of excitation and inhibition reported for the active states [12,32] towards inhibition. Thus, some neurons become more polarized, their firing threshold is harder to reach, no action potential is generated and as a chain reaction, the cortical network goes to the silent state. Additional intracortical mechanisms of active state termination may depend on synaptic properties. For instance, long-lasting active states produce short-term synaptic depression in excitatory synapses, consequentially the synaptic drive is unable to bring target cells to the firing threshold so the network goes in to the silent state [1,14]. This mechanism does not require an external regulator and can be responsible for triggering a local SO.

Differences between humans and animals

The six layers of the neocortex are each characteristically composed of specific neurons that connect with different cortical areas and the thalamus. Animal investigations reveal that cortical active states can start in any layer, but overall most commonly begin in layer 5. Layer 5 cells are the largest of the neocortex and exhibit the most extensive intracortical connectivity, and firing of layer 5 cells involves other cortical layers in ferrets [17], cats

Figure 2



Neuronal and muscle activities in neocortex during wake, slow-wave sleep and rapid-eye movement (REM) sleep. Three segments of simultaneously recorded neck muscle electromyogram (EMG), intracellular activities from area 7 (left hemisphere) cortical neuron, and area 7 (right hemisphere) and extracellular activities from local field potential (LFP) in a cat (modified from Ref. [1]).

[10] and rats [33]. Yet, a divergent picture arises from intracranial recordings in humans, as tested in epileptic patients: current source density and neuronal firing analyses suggest that active states often start from layer 3 [34,35]. There are several possible explanations for these differences between species: (a) Although data from healthy humans are lacking, the human epileptic brain may consist of reorganized connectivity and relatedly, the possible alteration of spatio-temporal involvement of cells entering the active state; (b) Reconstruction of pyramidal cells from resected cortical tissue suggests that in patients, the layer 5 pyramidal cells are typically smaller compared to pyramidal cells from layers 2–3 in the same patients [36], thus transmembrane currents from layer 2–3 neurons would be stronger; and (c) Layer 5 human pyramidal cells are considerably larger than layer 5 rat pyramidal cells, and due to their size, exhibit larger electrical compartmentalization that causes decreased sensing of dendritic activity at the level of soma [37] providing the possibility for distal dendrites to create strong excitatory currents and LFP signal without major impact at the level of neuronal soma.

Local and global waves

Silent states can be generated locally, if mediated by short-term synaptic depression due to local mechanisms, or globally, with the involvement of the subcortical drive of cortical interneurons. Wave detection within the frequency 0.1−4 Hz in high spatial resolution EEG recordings (high-density EEG, 256 channels) from healthy human adults suggests that the majority of detected scalp-SW affect ~20% of all electrodes [38]. Observations at younger ages support this regional restriction, such that a typical scalp-SW in healthy children involves on average ~15% of the electrodes in high-density EEG (128 electrodes), which would be considered a local scalp-SW [39••]. In line with this observation, intracranial LFP and multi-unit recordings from epileptic patients demonstrate that the majority (85%) of all cortical-SW are local [40], indicating that the global cortical-SW or scalp-SW only appear in a smaller fraction of occurrences. Additionally, data from intracellular recordings of anesthetized cats (suprasylvian gyrus, recorded at 12−15 mm distance, but receiving similar thalamic inputs) reveal that 80% of silent states coincide in time. In multi-site intracellular recordings (performed within 0.5 mm) almost all silent states occur simultaneously [10]. Considering these results in the context of a role of long-range projections of LP thalamic nucleus, we can conclude that the vast majority of sleep SO and SW are local also in animals [15,24,41].

Results from intracellular recordings in sleeping cats indicated the absence of neuronal firing during silent states, because neurons were hyperpolarized [8–10,13]. However, LFP and multi-unit recordings from the same electrodes show a strong reduction, but no complete absence of spikes during cortical-SW in humans [34,35,40], rats [42] and mice [43]. This suggests that in local cortical constellations, not all cells enter into silent states simultaneously. Furthermore, although it is commonly assumed that the cortical SO rhythm is absent during wake, sleep deprivation can trigger singular incidents of local cortical SW during wakefulness in rats [44]. During prolonged waking periods, it is likely that local cortical SO occurs as a result of synaptic short-term depression. Yet, it is unclear why the synaptic depression does not induce singular waking-SW during the normal (not extended) waking state. Several studies suggest that increased network activity reduces overall synaptic dynamics, as observed in cortical slices from ferrets and *in vivo* recordings in cats [45,46]. A possible explanation is that high cholinergic activity during waking reduces synaptic efficacy and leads to synaptic stabilization, as examined in mice, rats [47], and cats [48].

The onset of active states is triggered when the network is silent, and this condition experiences self-maintenance. Active states typically start in layer 5 cells and rapidly propagate to other cortical layers. Once initiated in one cortical location, the active states propagate across the cortical mantle. Studies in human [40] and mice [23,49] demonstrated that the individual active states can generally start at any location, but most often they begin in the frontal cortex, confirming the wave propagation activity across the cortical mantle as observed with scalp-SW in adult humans [38].

Sleep SO alters effective cortical connectivity

Because scalp-SW propagate across EEG channels, they are sometimes referred to as 'traveling waves' [38]. SW propagation patterns in humans are complex with signal dynamics involving convergence, divergence, and circulation [50]. Similar to SWA that accumulates and dissipates with homeostatic sleep pressure [51,52], traveling scalp-SW and cortical-SW also undergo across-night dynamics in human adults [40] and children [39••]. Dynamics of scalp-SW and cortical-SW are likely influenced by sleep-wake history (sleep homeostasis) and circadian timing and have been linked to neuronal connectivity in human adults [53••] and mice [22•].

Findings from experimental perturbations using Transcranial Magnetic Stimulation (TMS) have started to shed light on the relationship of SW with cortical connectivity. TMS is an established tool for assessing the interaction between cortical connectivity and consciousness in humans, which evokes EEG responses in targeted brain regions. Because TMS can elicit scalp-SW and cortical-SW in healthy humans and clinically diagnosed patients, investigating the role of natural SW in relation to cortical connectivity and states of consciousness is an important area of ongoing investigation [54].

Connectivity is quantified in manifold ways, for example, (a) Cortical complexity — capturing the interplay of both functionally segregated local areas, as well as their global integration during perception and behavior [55]; (b) Effective connectivity — the ability of a set of neuronal groups to causally affect the firing of other neuronal groups within a system [56]; or (c) Stability of connectivity — the dynamic (spatio-temporal) dimension of functional connectivity (temporal coactivation between brain regions), reflecting the time-varying signal propagation [57]. Elicited scalp-SW led to the discovery that with sleep onset, there is a breakdown in effective connectivity [58]. Interestingly and in contrast, cortical complexity does not greatly change across the wake period [53••,59]. Simultaneous EEG and functional Magnetic Resonance Imaging recordings support this observation, such that effective connectivity differs between sleep and wakefulness [60]. Specifically, in NREM sleep N2, connectivity is instable — suggestive of a redistribution of within-network and across-network information. In contrast, effective connectivity is stable in N3, portraying slow wave sleep as a relatively inactive condition with possibly more local integration [60]. Overall, insights are emerging from data on scalp-SW and cortical-SW dynamics indicating that during the transition to deeper sleep, a breakdown of neuronal connectivity occurs that gradually relates to dissipating consciousness.

Sleep-like events in the waking period

The idea of the clearly separable vigilance states of sleep and wakefulness has recently been challenged by data showing the occurrence of local SW in waking. As mentioned above, in freely behaving rats, after staying a long period in the awake state, cortical neurons can briefly go 'offline' as in sleep — this is reflected locally in one cortical area but not in others [44]. Investigations with scalp EEG in humans support the concept that local sleep-like events can be detected (particularly extended) during waking periods: Local sleep-like events were found to intrude on wakefulness [61]. This investigation quantified the cortical size of local events in children's waking EEG by means of the number of electrodes involved in theta events. Approximately 6%–15% scalp electrodes were involved in a local sleep-like event for detection windows of 20−100 ms [61]. This study further revealed that theta waves (6−8 Hz) become more widespread in the evening and are associated with slower reaction times – suggesting specifically that theta waves are markers of local sleep in humans. Thus, while local sleep represents neuronal off periods, the synchronization of off periods can enter the spatial domain: OFF periods can propagate across the cortical mantle and become visible as traveling scalp-SW in sleep, or traveling theta waves during extended waking. Although these attempts take place in the time domain of seconds (off-states during waking in rats 50−100 ms [44], and theta waves in children 20−100 ms [61]), the transition from wakefulness to sleep includes modifications in several dimensions: alertness, neuronal connectivity and behavioral performance [53••].

Local aspects of sleep yield similar electrographic patterns in mammals, reptiles [62], birds [63], and fish [64] and further revolutionize current understanding of brain states. In other words, it is now recognized that variability exists across cortical regions during wakefulness, NREM sleep and REM sleep states. For example, SW — classically the most typical characteristic of NREM sleep — was recently discovered to also exist in REM sleep of mice and men [65–67]. Thus, local SW also appear in REM sleep [66,67] and wakefulness.

Although local aspects of sleep have been primarily investigated as cortical manifestations of NREM sleep, research in birds indicates that muscle tone during REM sleep also appears to be regulated at a local level [68]. One investigation demonstrated that REM sleep-related reductions in skeletal muscle tone appear largely restricted to muscles involved in head posture maintenance. Relatedly, it was proposed that muscle atonia and REM sleep are mediated by the brainstem, while SO in NREM sleep is detected in the hyperpallium (primary visual area in ostriches) [68]. Additionally, the findings in birds further imply a prominent role of thalamic input layers in the initiation of propagating SO. Further, SO propagation varies across layers of avian hyperpallium (the primary visual area), such that SO first occur in, then propagate through and outward from thalamic input layers.

The evolution of SW and SO across species unravels from 'bottom-up' a reconstruction of the neurophysiology of our primary behavioral states, including specifically the transitions manifested in local sleep-like events. Yet, it is largely unknown to what extent our living context influences local sleep-like events from 'top-down'. While the sleep/wake routine of our society largely defines a 24 hour rhythm, astronauts witness short sunrises several times a day, as the International Space Station orbits earth every 90 min. Even though an 'artificial' 24 hour routine is created, a majority of crew members suffer from low sleep quality. Thus, markers of sleep pressure were examined in astronauts who were on a 6-month space mission using high-density EEG [69]. Using theta frequency, local sleep-like events were detected in the wake period. Interestingly, these events occurred across more widespread cortical areas when humans were in space than on earth ($4.06 \pm 0.66\%$ and $3.26 \pm 0.66\%$ increase of electrodes involved in a sleep-like event [69]). Average 'globality' varied between 15 to 25% of scalp electrodes. This research also specifically linked wave globalization to the slowing of behavioral reaction time, thereby suggesting a link between cortical synchronization of SO and behavioral function.

Function of propagating SO

SO are universal across species. SO expand from micro to macro scales, and were described as the 'default activity' of the cortical network [15], based on their continuing expression in situations of physical or functional disconnection of the cortex (for a comprehensive multi-species review see Ref. [70•]). It is not clear whether SO activity *per se* serves a function regarding the neuronal network or behavior; however, the following proposals have been made supporting such a concept.

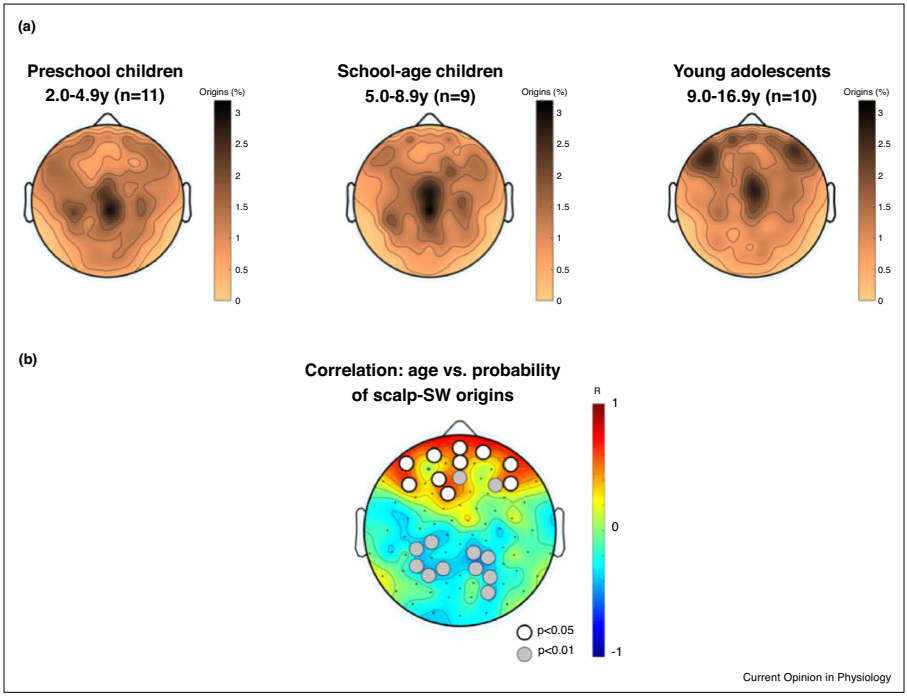
One notion is that scalp-SW reflect neuronal activity and that their propagation dynamics mirror brain connectivity in developing [71] and adult humans [72]. Accordingly, the non-invasive assessment of scalp-SW provides insight into cortico-cortical interactions, excitability of the cortex and a link to behavioral states. Indeed, intracranial SW propagation has been associated with consciousness (in epilepsy patients) [73]. This linkage is complex and, for instance, includes the possibility that specific long-range or short-range SW dynamics relating to particular states of vigilance and behavior, as suggested from research in freely behaving rats [74•]. Similarly, altered spatio-temporal scalp-SW dynamics negatively affect performance in vigilance tests (humans) [53••,75]. In this context, scalp-SW were suggested to represent a form of 'neuronal tiredness'. The concept has thus emerged that scalp-SWA goes beyond the pure reflection of neuronal dynamics and in addition may directly alter the neuronal network and, as a consequence, behavioral activity.

Another consideration is that the propagation of SW modifies neuronal connections. In particular, SW have been linked to memory consolidation processes during sleep (for reviews see Refs. [76,77]. Enhancing SW using electrical stimulation appears to improve hippocampal-dependent memory performance in both humans [78] and rats [79]. A recent study demonstrated that electrical field stimulation induced a transformation of SW dynamics in mice and therefore proposed this method for manipulating memory processes [80•]. Mechanistic underpinnings of the SW-memory link may include high-frequency signal propagation from cortex to hippocampus during wakefulness, and low-frequency activity in the opposite direction during slow wave sleep. Recent findings corroborate such a state-dependent turnaround of cortical–hippocampal communication in humans [81]. The pattern of SW-propagation and SO-propagation may determine the synaptic strengths between neurons, as addressed in a thalamocortical network model [82••]. Synaptic plasticity, cortical-SW, and phasic hippocampal discharges possibly trigger some form of plasticity during SO that contributes to sleep-dependent memory consolidation [82••]. Furthermore, relevant to this context (at least in humans) is the balance between the circadian and homeostatic drive for sleep [83], as well as wave-specificity, such that different observations exist for local or global SW. For instance, using scalp-SW trough traveling profiles in healthy human subjects, only global SW moved anteriorly to posteriorly, in comparison with local and frontal SW [84•]. Additionally, global scalp-SW revealed also stronger coupling with fast spindles. Hence, experimental research is needed to uncover which types of scalp-SW are causally relevant for cognition and memory processes, specifically because the exact mechanisms of sleep-dependent synaptic modifications (plasticity) is a matter of debate [1,85,86].

A further viewpoint can be taken from an evolutionary perspective. The mammalian cortex is viewed as the zenith of neuronal evolution, yet how its laminar cytoarchitecture mediates complex cognition remains poorly understood. Comparisons of sleep-related neuronal activity with non-mammalian groups lacking laminar cytoarchitecture provide insight into neocortex functioning [63]. The SO also exists in birds, yet interestingly, it propagates in neuronal activity of complex three-dimensional trails [63]. This represents a contrast to the two-dimensional SO propagation observed in mammals with laminar organization of the neocortex. Accordingly, it was proposed that the non-laminar, nuclear neuronal cytoarchitecture in birds may have emerged from computational properties of this specific dimensional geometry. Examinations in Nile crocodiles [87] also suggest that the SO propagation may connect to the evolutionary elaboration of nuclear structures, which may also relate to the advancement of complex cognition.

Building on these concepts, a further discussion emerges suggesting that propagating SO and SW possibly serve an active role in the modification of processes of brain development and brain evolution. To test whether the maturation of synaptic dynamics is related to sleep and wake states, juvenile mice served as a model system for human adolescence development. In line with dynamics in adult animals, cortical spines in 1 month old mice were increased during waking and decreased during sleep [88]. However, only the developing mice revealed an overall net increase in spine density. In addition to this maturation in terms of spines and SO, maturation dynamics also happen on a topographical perspective. In humans, the distribution of scalp-SWA changes considerably during childhood. These changes in the sleep EEG mirror cortical anatomical processes by shifting from predominantly posterior to anterior regions [89]. Furthermore, propagation parameters of scalp-SW change during childhood, as we reported in two recent investigations using high-density sleep EEG in children aged 2–16 years [39••,71]. This study focused on scalp-SW propagation properties including distance proliferated, propagation speed, and cortical involvement (number of channels, in which a SW is detected). We found that scalp-SW propagation undergoes age-specific changes that are associated with white matter microstructure (brain myelin) [71]. Specifically, across development, scalp-SW

Figure 3



(a) Topographical distribution of probability of origins of scalp-SW in 30 healthy children. All-night high-density EEG at-home assessments were performed in 11 preschool children (2.0–4.9 years), 9 school-age children (5.0–8.9 years) and 10 adolescents (9.0–16.9 years). Dark colors refer to high probability of origins at the indicated electrode, while light colors refer to low probability of scalp-SW origins. Data processing entailed band-pass filtering (0.5–40 Hz), rejection of artifact-containing channels and re-referencing to mastoids. A previously published algorithm was used for wave detection and computation of propagation delay, for details see Refs. [39••,71]. **(b)** Linear correlations of age with origin of scalp-SW. Pearson correlations were performed at each electrode. Red indicates significant positive correlation with age, while blue indicates negative correlation ($p < 0.05$). The figure shows that with increasing age across childhood, scalp-SW are more likely to originate in frontal electrodes and less likely to originate in parietal channels.

propagation distance increases, which is associated with myelination of the corpus callosum. Propagating speed and cortical involvement also relate to myelination of the superior longitudinal fascicle. Furthermore, across-night dynamics of scalp-SW propagation are specific to age, possibly reflecting heightened plasticity in neuronal networks specific to sensitive developmental periods [39••]. In preschool children propagation distance decreases across the night, while this decrease is neither found at school-age nor at adolescence. Interestingly, even though cortical involvement of the propagating scalp-SW appears relatively stable across age, it undergoes a homeostatic decrease across sleep. Our novel data presented here indicate that scalp-SW originate most often in centro-parietal areas in younger children, whereas when youth are approaching adolescence, frontal origins are more frequently observed (Figure 3).

Our findings address spatio-temporal scalp-SW dynamics and demonstrate the maturation from central towards frontal scalp-SW onset in developing humans. In human adults a frontal predominance of scalp-SW onset is typically observed [38] — a pattern that is highly reproducible across different recording nights, that is, indicating high stability within participants. From a specific cortical focus location, scalp-SW travel across the cortex [38,90] in

mostly an anterior-to-posterior direction, yet with complex pattern variation [50,91]. The transformation from child to adult SW patterns thus highlights a connection between SW propagation and brain maturation and represents the potential of detecting deviations of developing cortical networks with sleep high-density EEG.

Data from developing rodents come from only a handful of studies; however, to our knowledge, findings in rodents generally align with regional scalp-SW dynamics reported in humans. Similar to humans [38,40,72], individual active states in mice most often start in the frontal cortex from which they propagate in anteroposterior/lateral direction over the cortex [23,49]. Multielectrode arrays reduce the past limitation of poor spatial resolution signal from mice and rats and data from such studies indicate topographical differences in mice, such that faster DOWN-to-UP state transitions, higher firing rate during UP states, and more regular cycles are observed in the prefrontal cortex [49]. Triggering SW with stimulation in adult rats revealed likewise predominantly early cortical-SW propagation from frontal regions, some isolated SW also originated from posterior areas [42]. Complementary knowledge from reports in mice indicates the subcortical control of cortical-SW: activity in centro-medial neurons in the thalamus precedes the UP states in the cingulate cortex [22•]. Future studies with nonhuman primates that use novel techniques of imaging [92,93] or optogenetics [94] will allow to characterize primate SO thoroughly. Furthermore, they will provide direct testing of the functional role of SO and SW, as proposed in the context of propagating waves during waking [76].

It is possible that behavioral correlates of SO and SW maturation exist, for instance those involving the motor or cognitive domains. Accordingly, more frontalized scalp-SW onset may relate to advanced cognitive skills. We may further speculate that spurts of maturation in motor skills may be connected to increased probability of scalp-SW onset in the motor cortex, representative of critical developmental periods. Repeated longitudinal (within-subject) assessments across the time period of months to years when the specific skill maturation occurs are needed to capture such transitions. Furthermore, behavioral tasks known to specifically involve the cortical regions are needed (see Ref. [95]), and the involvement of white matter microstructure (myelin) in performance should be considered [71,96••,97].

Conclusions

In summary, the recent trends in the field of spatio-temporal properties of SO and scalp-SW can be highlighted as follows:

- Scalp-SW during sleep reflect the SO rhythm of hyperpolarization and silence of cortical neurons that occur synchronously across cortical regions. SO are controlled by subcortical structures involving the thalamus. While approximately 80% SO and scalp-SW are local, about 20% appear global.
- Scalp-SW propagate across the cortical mantle in complex patterns. They originate mostly from the frontal cortex in adults, but from centro-parietal regions in young children.
- Propagation patterns undergo across-night dynamics. Neuronal organization underlying SW and SO dynamics is connected to vigilance states and degrees of consciousness.
- The function of isolated SW and SO remains unclear but they may (a) purely reflect neuronal activity; (b) modify neuronal connections, affect network connectivity, and maintain cognitive and memory processes; (c) be connected to the evolutionary elaboration of nuclear structures and complex cognition; and (d) actively convey modification processes of the developing brain.

Conflict of interest statement

BR is supported by Philips Healthcare, no other conflicts of interest are declared.

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7

DISCUSSION

All you really need to know for the moment is that the universe is a lot more complicated than you might think, even if you start from a position of thinking it's pretty damn complicated in the first place.

— Douglas Adams

This thesis investigated how sleep matures in infancy and beyond and how its maturation relates to gut bacteria and behavioral development. I studied sleep using actigraphy and 24-h-diaries in a large cohort of 162 babies, as well as EEG in a smaller subset of 33 infants. To gain reliable sleep estimates, I first improved and standardized the analysis pipeline to extract sleep estimates from actigraphy for this age group. Then I analyzed how sleep matures in infancy, and related it to gut bacteria and behavioral maturation. Additionally I used an existing cross-sectional dataset of sleep EEG in 2 - 17 year olds to look at spatio-temporal aspects of traveling slow waves.

In Article 1, we did a systematic review of the literature to quantify adherence to reporting standards in actigraphy since Meltzer et al. suggested reporting standards in 2012. While reporting improved in some areas, we demonstrated that other areas were still lacking, especially details regarding analysis algorithms, artifact identification, and definitions of used sleep variables. Additionally, we revealed that while most studies record activity patterns continuously, only 1/5 of the studies doing so characterized daytime sleep. Since daytime sleep makes up a considerable amount of sleep in infancy, it is essential to also include it in analyses. We issued some recommendation on actigraphy recording and reporting including use of devices with access to raw data, continuous recording and analysis across 24 h in infants.

In Article 2, we addressed the issue that a large source of variability in actigrapy resarch comes from the use of different analysis algorithms to estimate sleep-wake patterns from movement. We compared two commonly used actigraphy algorithms (Sadeh and Oakley/Respironics) and the sleep-

estimates they produce. We found that the two algorithms provide very different results so that, e.g., sleep duration estimates vary on average by 4.5 hours per 24-hours. This difference can bias the establishment of norm values for sleep behavior in infancy. We proposed a pipeline to analyze actigraphy data that reduces the differences between algorithms and improves agreement with a 24-h diary: the average sleep duration estimates only vary by less than 15 minutes. Applying this pipeline will help generate sleep-wake estimates that are more comparable across studies, even if different devices and algorithms are used.

In Article 3, we standardized the next step in actigraphy analysis: selecting sleep variables to calculate the sleep-wake estimates. We applied a principal component analysis to our infant sleep data set to extract five underlying sleep composites from 48 sleep variables, which accurately reflect sleep-wake patterns. *Sleep Activity* reflects nighttime awakenings and activity during sleep, *Sleep Night* reflects nighttime sleep duration, Sleep Day reflects daytime sleep duration and regularity, *Sleep Timing* reflects times of going to sleep and getting up, and *Sleep Variability* reflects the variability of *Sleep Timing* and *Sleep Night*. Using these sleep composites, we made some exciting discoveries about the maturation of sleep in infancy: 1) We found that sleep was variable across infancy, not only between infants but also within infants, with only *Sleep Timing* staying relatively stable across the first year (Roehrs et al., 2006; van den Berg et al., 2009). 2) We found that *Sleep Activity* showed reliable differences between male and female infants, with boys having more awakenings than girls. This difference continues throughout the whole lifespan. 3) Very interestingly, we found that *Sleep Day* showed a higher positively correlation with total sleep duration across 24 h than *Sleep Night*. This suggests that it is not necessarily a trade off between daytime and nighttime sleep in early infancy. Rather, daytime sleep is a marker of general sleep need. We also found that more daytime sleep was negatively correlated with behavioral development at the same age, suggesting it might be a useful marker of overall developmental status.

In Article 4, we investigated our core research question if sleep and gut bacteria are linked in infancy and how they relate to development. We used a cohort of 162 infants that we measured at 3, 6, and 12 months of age with a behavioral follow up at 24 months. We analyzed the maturation of gut bacteria in infants and found that our sample agreed well with

previous literature: alpha diversity increased drastically, and beta diversity decreased across infancy. The composition shifted from *Bifidobacteria* as the most abundant species to *Bacteroides*. We found that infants cluster into two enterotypes depending on *Bifidobacteria* and *Bacteroides* abundance. Most infants were in the *Bifidobacteria* subtype at 3 months but switched to the *Bacteroides* subtype with later age. We examined the associations between 3 markers of gut bacteria with sleep: alpha diversity, enterotype and bacterial maturity. Using the sleep composites established in Article 3, we found that alpha diversity was associated with *Sleep Day*, especially at 3 months of age. Infants with higher diversity (more mature) were also more mature in their sleep behavior (less day time sleep). Furthermore, we found that *Sleep Activity* was related to enterotype at 12 months of age. Infants who were still in the *Bifidobacterium* enterotype showed more awakenings at night. Lastly, we showed an association of bacterial maturity and *Sleep Activity*. However, contrary to our expectations, the infants with more mature gut bacteria showed more *Sleep Activity*. Furthermore, we found interesting associations between both sleep and gut bacteria and development. These associations were the strongest at 3 months, suggesting an early sensitive period.

In Article 5, we expanded beyond infancy and examined the spatio-temporal properties of slow waves across childhood and adolescence (2 - 17 years). Specifically, we examined the across-night changes of slow wave propagation, including propagation distance, traveling speed, and cortical involvement (dispersion across the scalp). We found that propagation distance increased with age. Interestingly, propagation distance decreased across the night but only in children younger than 5. This age-specific effect shows a potentially sensitive period for brain maturation. Furthermore, we found that slow wave cortical involvement decreased across the night in all age groups. Slow wave speed showed no apparent change across age or the night.

In Article 6, we reviewed the literature on the relationship between slow wave activity, scalp slow waves, and intracellular slow oscillation. We proposed standardized terminology to distinguish between these three concepts. We presented new data showing that the slow wave origins change across development: in children, the slow waves originate from central regions, while in adolescents, most slow waves originate from frontal areas.

Slow waves reflect maturational processes, but we proposed that slow waves might additionally modify neural connections both during learning and memory and across development.

In the following section, I will discuss methodological recommendations and some of the most notable findings from the articles. Additionally, I will highlight potential future directions for developmental sleep research.

7.1 METHODOLOGICAL RECOMMENDATIONS

7.1.1 *How do we best measure sleep in infancy?*

Using actigraphy to measure infant sleep is a feasible, cost-effective approach. The benefits of being able to measure sleep in natural environments for prolonged periods are tremendous. The variability that we have found in infant sleep, both between and within infants, underscores the need to measure sleep across multiple days and multiple time points to measure each infant accurately. For the moment, actigraphy is the only practical solution to generate this kind of data. While videosomnography can also be recorded across extended periods, it is often not ideal for measuring daytime sleep periods because infants tend to nap in different locations. As we have shown, daytime sleep is a crucial aspect of infant sleep and it is fundamental to include it in sleep studies. While similar data can be collected using diaries alone, this is prone to be biased by parental perceptions. For example, the increased infant night wakings when co-sleeping is only seen in subjective and not objective data, likely because parents only notice brief awakenings when they sleep in the same room (Volkovich et al., 2015). Parents also sometimes forget to note items such as awakenings (Müller et al., 2011). Therefore, the combination of 24-h-diaries and actigraphy is best suited to collect habitual sleep data in infants.

While using a sleep EEG is the gold standard for sleep research, it is not practical to use EEG to collect habitual sleep data in infants due to the expenses of both time and money. Furthermore, with advancing technology and analysis approaches, we can gain more information from actigraphy than just sleep versus wake state. It has been shown that a similar pattern to sleep stages (as scored by EEG) can be seen in actigraphy when assessing periods of motor inactivity (Locomotor inactivity during sleep,

LIDS, Winnebeck et al., 2018). Preliminary analyses with our infant cohort and the LIDS methods confirmed shorter cycles in infants, which mirrors the sleep stages measured with EEG (Winnebeck, Schoch, et al., in prep). However, EEG is still invaluable to gain insights which are not accessible through actigraphy – sleep is not just a global state but varies locally across the brain. Recordings with hdEEG are needed to detect local sleep differences. As these local differences mirror brain development (Kurth et al., 2010), sleep EEG can give us insight into brain maturation. The sleep EEG can even be used as markers for later development (LeBourgeois et al., 2019). Therefore, actigraphy and EEG should be seen as complementary approaches. The best choice depends on the focus of the study. Actigraphy is the recommended method to measure habitual sleep in infants, while EEG is preferred to measure sleep stages and gain insights into brain maturation.

7.1.2 *The future of infant actigraphy sleep research*

A significant part of my thesis was devoted to improving research standards for actigraphy sleep research in infants. We suggested detailed methodological reporting, which increases the comparability between studies and makes the inclusion of studies into meta-analyses easier (Schoch, Kurth, et al., 2020). Additionally, we have developed a pipeline to make estimates across different sleep algorithms more comparable (Schoch, Jenni, et al., 2019). However, we only had data available from one actigraph; therefore, we will need to validate this pipeline in data collected with other actigraphs. We also focused on the two most commonly used algorithms; our results might not generalize to other algorithms. Therefore, we suggest that researchers examine their actigraphy-based sleep data with at least two algorithms to determine how dependent their results are on the algorithm they chose. If they find large differences, we recommend using some or all of the adjustments we suggested in our pipeline to make their results more comparable.

Lastly, we applied a principal component approach to infant actigraphy sleep data and found five underlying sleep composites (Schoch, Huber, et al., 2020). We recommend that researchers use the same principal component approach or use single sleep variables with high loadings onto these composites to standardize sleep variables across different studies. We only

used this approach on data with one actigraph and algorithm; therefore, future studies will need to confirm that the same structure sleep composites are found with different methods. However, the fact that we found the same underlying composites as Staples et al. (2019) reported with young children using a different actigraph and different sleep variables gives us confidence that similar composites will be found regardless of methods. Ideally, actigraphy would develop the same type of standardization as is present in sleep EEG research. The development of a manual that includes recommendations on everything from recording to analysis would be helpful.

A next step would be to develop a Matlab or python toolbox or R package to analyze infant actigraphy data with different algorithms, the adjustments we proposed, and incorporating a 24-h diary. Ideally, this would also automatically calculate the sleep composites. A toolbox like this would help researchers that do not have the time or ability to code and develop their own analysis to adopt an improved analysis pipeline. In the last years, several packages and toolboxes have been developed e.g., nparACT (Blume et al., 2016) or pyacthigraphy (Hammad et al., 2020). However, these seem to be aimed more towards adult actigraphy data. Infant sleep analysis needs some special consideration, e.g. due to external motion (by caregivers) and unique algorithms used for infants, because they show more activity during sleep (Tonetti et al., 2017).

Another future development for actigraphy is hopefully that collaborative big data approaches are embraced. Due to the large variability and instability of sleep behavior in infancy, we will need an extensive database across many infants, countries, and measurement devices. Such a database would be beneficial for many endeavors: 1) to establish normative sleep data, 2) to understand the influence of different actigraphs and algorithms on estimated sleep-wake behavior, 3) to run meta-analyses on the relationship of infant sleep with later outcomes and hopefully clear up the previously contradictory results found within objective data (Sadeh et al., 2014). I hope that the field will embrace open and collaborative science to gain more insights and hopefully improve the lives of infants and their parents.

7.1.3 *The future of infant EEG sleep research*

While EEG sleep research is more standardized across research groups than actigraphy, there are still areas that lack standardization. The classification of sleep stages from EEG is essential to quantify sleep architecture in sleep research and sleep medicine. However, visual sleep stage scoring is a time-consuming process that is prone to human error. Differences in scoring emerge, especially between laboratories (Norman et al., 2000). A particular challenge is scoring the infant EEG, as several features used to distinguish sleep stages are still in development during infancy. Recent advances leverage deep learning techniques for automatic sleep stage scoring, but most algorithms are trained on a few EEG channels and focus on adult sleep staging (see Fiorillo et al., 2019 for review). We examined the feasibility of using deep learning (Long Short-Term Memory network) to automate infant sleep scoring. Additionally, we investigated the effect of including more channels on scoring accuracy (Schoch, Verzhbinsky, et al., 2019). We found that it is feasible to train a network on EEG data, but the sample size of n = 31 infants is too small to have a network that generalizes to datasets it has not previously encountered. Additionally, we found that including more electrodes improved the network's accuracy, but effects leveled off after 88 electrodes. A multi-laboratory big data approach would be beneficial here. Ideally, we will train a network on data from different laboratories, EEG systems, and scorers. While setting up the original collaboration might be time-intensive, this approach will save time in the long run while simultaneously improving reproducibility across laboratories. Furthermore, it will be interesting to apply novel approaches like microstates on infant sleep EEG data. Microstates refer to EEG periods of milliseconds to seconds, allowing for a much greater resolution and dynamic analyses than the previously applied 20/30s (T. Koenig et al., 1999). A better resolution can also be achieved spatially. Using hdEEG is beneficial because it gives insights into the topographical distribution of sleep states. Evidence has accumulated that sleep has many local aspects despite being a global phenomenon (see Siclari and Tononi, 2017 for review). Applying sleep scoring globally, therefore, neglects these local differences. Advances to score automatically could enable the scoring of each electrode separately and give us more insights into the local regulation and maturation of sleep across development.

7.2 SLEEP MATURATION

7.2.1 *Daytime sleep – a marker for maturational status?*

Sleep Day has emerged across several of our analyses as a potential marker for development. Firstly, it shows some of the largest changes across infancy, with daytime sleep duration decreasing by 40% from 3 to 12 months (average daytime sleep at 3 months: 4.1 hours, at 12 months: 2.5 hours) (Schoch, Huber, et al., 2020). Secondly, we found that across all assessment time points, *Sleep Day* was associated with behavioral development, with infants that slept less during the day scoring higher on overall development. This effect was most substantial at 3 months and became smaller across development (Schoch, Huber, et al., 2020). Third, very interestingly, *Sleep Day* at 12 months negatively predicted gross motor development at 24 months. Thus far, studies on sleep and motor development seem to not have focused on daytime sleep (Anders & Keener, 1985; A. Scher, 2005; M. S. Scher et al., 1996). However, the amount of wakefulness after birth is associated with motor development (Anders & Keener, 1985). The highest (negative) loading factor of *Sleep Day* is the longest wake period. Therefore this might reflect the same underlying effect, potentially that more time awake enables more learning possibilities or that the ability to sustain longer wake periods reflects a more mature neurological system. Fourth, we found that *Sleep Day* is associated with the gut bacteria diversity, precisely with alpha diversity across all time points, most notably at 3 months of age (Schoch et al., 2021). Fifth, when examining the association between EEG power bands during NREM sleep and the five sleep composites, we found that *Sleep Day* was positively associated with absolute slow wave activity (1 - 4.5 Hz, $r = 0.48$, $p = 0.0067$, partial correlation corrected for age at measurement). No associations were found with theta (4.75 - 7.75 Hz) or sigma (10 - 15 Hz) frequency (Schoch et al., in preparation).

To further explore these associations, we looked at the topographical distributions of the frequency bands in association with the sleep composites (cluster-corrected and Bonferroni correction of p-values). We found a cluster of electrodes in the parietal region that showed increased slow wave activity in infants with more daytime sleep. Additionally, two electrodes in the left temporal region also showed increased slow wave activity (see Figure 9). The positive association of daytime sleep and slow wave activity,

a marker of sleep pressure, might seem counterintuitive at first. However, it is reasonable when framing *Sleep Day* as a marker of general maturation. Likely the increased daytime sleep is not only due to less nighttime sleep but also due to a general immaturity of the neurophysiological system. This immaturity means that the infant cannot sustain wakefulness for a prolonged period, and sleep pressure accumulates faster. These findings fit with what has previously been reported in toddlers where slow wave activity was higher in a nap in the late afternoon compared to morning. This increase was only seen in children at 2 and 3 but not at 5 years old (Kurth et al., 2016). Therefore, infants sleep during the day not because they do not sleep at night, but because sleep pressure accumulates quicker. The findings of Kurdziel et al. (2013), which show improved memory performance after a nap in habitual napping toddlers, seem to agree with this. However, another study found better memory in habitual nappers versus non-habitual nappers (Lukowski & Milojevich, 2013). One should also note that a previous study suggested that theta, not slow wave activity is a marker of sleep pressure in infants, as it shows a pattern of dissipation across the night (Jenni et al., 2004). However, our slow wave activity included higher frequencies (1 - 4.5 Hz, compared to 0.75 - 1.75 Hz), which potentially show a different homeostatic pattern. As we only recorded a maximum of the first two hours of sleep, we cannot examine the dissipation patterns of slow wave activity and theta activity across the night.

Interestingly, *Sleep Activity* shows a higher positive correlation with *Sleep Day* than negative correlation with *Sleep Night*. One possibility is that infants with more awakenings at night have to catch up on sleep during the day. Another potential explanation could be that the ability to sustain one state (sleep or wake) continuously is dependent on the maturation of the brain. Therefore infants with long daytime sleep would have a general reduced ability to sustain one state. However, what speaks against this is that Longest Wake Period during the day and Longest Sleep Period during the night are not significantly associated in our sample (Schoch et al., in prep).

While there is a negative association between *Sleep Day* and *Sleep Night*, it is smaller than might be expected. Together with the finding that *Sleep Day* shows a stronger association with sleep across 24 h than *Sleep Night*, this hints towards *Sleep Day* being associated with general sleep pressure. Our

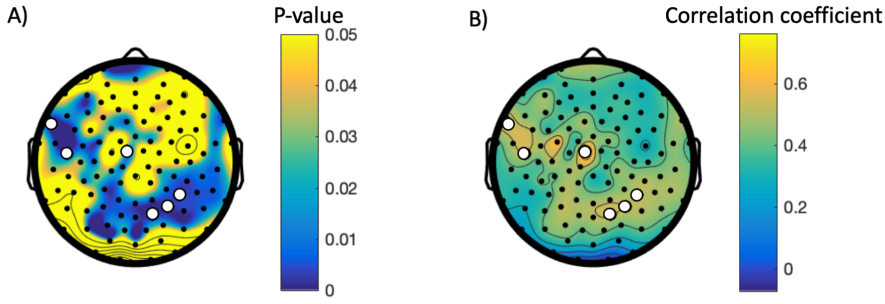


FIGURE 9: Association between absolute slow wave activity (1 - 4.5 Hz) and Sleep Day. A) p-value distribution, blue areas show low p-values. White dots mark significant electrodes ($p < 0.0033$). B) R value distribution, high correlations are shown in yellow and orange. White dots mark electrodes with significant correlations.

findings also highlight that daytime sleep should not be ignored in actigraphy studies. More research will be needed to establish the validity of *Sleep Day* as a maturational marker, but findings across behavioral maturation (Schoch, Huber, et al., 2020), gut bacteria maturation (Article 5), and sleep EEG activity (Schoch et al., in preparation) are providing the first evidence for this. Future research could use the Bayley Scales of Infant and Toddler Development to validate the link with behavioral development using a trained scorer as the Ages and Stages questionnaires (Squires et al., 1995), rely on parents' ratings (Bayley, 2006). Additionally, studies should investigate if early prolonged daytime sleep is associated with developmental disorders.

7.2.2 *Sleep Timing — intrinsic versus extrinsic factors?*

Sleep Timing reflects the average bed and wake times of an infant (higher scores show later bed and wake times). Infant sleep timing is interesting because it is very stable across infancy, despite the shift of bedtimes by nearly an hour (mean bedtime 21:20 at 3 months, 20:30 at 12 months; Schoch, Huber, et al., 2020). Unlike other sleep composites, the timing of sleep is most easily influenced by parents. When infants can only sleep through short periods, nighttime sleep is likely aligned with later sleep to allow

the parents to sleep simultaneously. Around 6 months after giving birth, most mothers in our sample returned to work, and the children are cared for externally for some days of the week. One reason for the shift to earlier timing from 3 to 6 months could align with the earlier rise times required for daycare and work. Not surprisingly, we found that the infant's sleep timing is linked to parental habitual bedtimes (Schoch, Huber, et al., 2020). However, parental bedtimes only explain a small amount of the stability that we find in *Sleep Timing*. Other parental factors likely influence sleep timing stability, such as parents wanting to keep a regular sleep schedule or aligning the sleep schedule with a sibling's schedule.

Next to the extrinsic factors, it is also possible that there are intrinsic factors of the infant already present at such a young age, i.e. an early chronotype. As chronotypes are influenced by genetics (heritability of around 40 - 50%, von Schantz et al., 2015), this could explain parts of the association between parental and infant sleep times. Interestingly, we found a small, albeit not significant, association between *Sleep Timing* and theta power ($r = 0.31$, $p = 0.09$). When we looked at the topographical association between theta activity and *Sleep Timing*, a cluster of central electrodes showed a significant positive association with *Sleep Timing* (Figure 10). Therefore, infants that generally have later bedtimes show more theta power in central regions. As discussed previously, Jenni et al. (2004) suggested that theta activity is a marker of sleep pressure in infants. We scheduled all our EEG measurements at infant habitual bedtimes. Therefore this finding should not be a measurement artifact. The greater theta activity in infants with late sleep timing could reflect higher sleep pressure. One hypothesis is that infants with later sleep timing have a mismatch between their circadian timing and their bedtimes, resulting in higher sleep pressure. In this case, the *Sleep Timing* would be guided by extrinsic factors. However, it is also possible that the increase in theta activity relates to an intrinsic factor that influences *Sleep Timing*. Central theta at 3 months has been reported to be lower in preterm than in term-born infants (Guyer et al., 2019). In contrast, topographical distribution pattern of theta at 6 months old are more similar to the preterm infants than the term-born infants at 3 months (see Figure 6), with maxima in both central and occipital regions. At 2-8 years, this bi-modal distribution persists, with the occipital areas showing the maximal power (Kurth et al., 2010). Therefore, higher theta activity within central regions could be less mature, and this could be associated with the later

Sleep Timing.

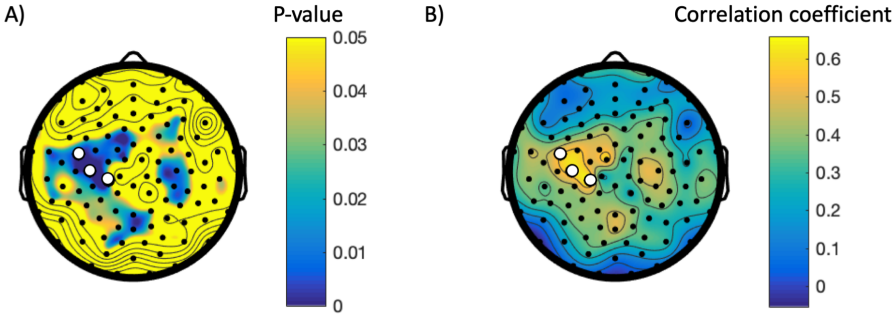


FIGURE 10: Association between absolute theta activity (4.75 - 7.75 Hz) and Sleep Timing. A) p-value distribution, blue areas show low p-values. White dots mark significant electrodes. B) R value distribution, high correlations are shown in yellow and orange. White dots mark electrodes with significant correlations.

We found that *Sleep Timing* was associated with behavioral development, with later sleep times at 6 months predicting both lower personal social and overall developmental scores (Schoch et al., 2021). This is similar to what was reported previously (Mindell et al., 2017). This finding could be explained by both the potential mismatch between circadian and extrinsic times and late *Sleep Timing* reflecting a generally heightened immaturity. Surprisingly, however, later *Sleep Timing* at 3 months, was associated positively with gross motor development at 6 months. But at 6 months, later *Sleep Timing* was associated with lower developmental scores at 12 months. Future studies will need to disentangle the extrinsic and intrinsic aspects of *Sleep Timing* in infancy. The next step could be to investigate the parents' attitude towards timing their child's sleep and how that relates to actual bedtimes and theta activity during sleep.

Although a subset of gut bacteria are known to have their own rhythms (not unlike humans) (Thaiss et al., 2014), we did not find any associations between *Sleep Timing* and our three gut bacteria markers. However, we did not include any markers that specifically measured gut bacteria rhythmicity because we only analyzed one stool sample per infant. Therefore, it will be

interesting to explore this in a further study. Ideally, such a study would include a higher sampling frequency of stools from single infants.

7.2.3 *Sleep Activity - change from functional to dysfunctional?*

Sleep Activity is the second aspect that shows the highest changes across the first year of life - wake after sleep onset (WASO) changes by 65% from 3 to 12 months (mean WASO at 3 months 70 minutes, mean WASO at 12 months 25 minutes). Awakenings during the night can be an enormous burden on parents. The night awakenings are one main reason for decreased sleep duration in parents - an effect that extends until 6 years after birth (Richter et al., 2019). Night wakings are a strong predictor of whether parents will report their infant's sleep as a problem, which is subsequently negatively linked to parent mental health (Hiscock & Wake, 2001). However, night wakings in infancy are normal. At 3 and 6 months, no infants slept through the night in all recording days. At 12 months, less than 5% of infants slept through all assessment nights without any awakenings. Early on, as infants get breastfed, night wakings have a functional component. The infant's stomach has a low capacity, and therefore the infant needs frequent feeding, and thus frequent wakings. Later on, the stomach capacity grows, and therefore feeding frequency decreases. However, if breastfeeding continues for longer, night wakings might continue (Elias et al., 1986).

We do not find any evidence that sleep awakenings at 3 and 6 months are associated with worse behavioral, EEG, or gut bacterial outcomes, suggesting that night wakings in early development are expected. Surprisingly, we found positive associations with early *Sleep Activity* and outcomes. At 3 months, more *Sleep Activity* was associated with higher personal social development. Furthermore, *Sleep Activity* at 3 months positively predicted personal social developments at 6 months. At 6 months *Sleep Activity* was associated with a more mature gut bacterial profile. One conceivable reason why *Sleep Activity* could have beneficial effects is that as sleep-wake patterns are scored via increased activity, it potentially also captures motor activity during sleep. During sleep, the activity level is higher at the beginning of life and decreases with age (Tonetti et al., 2017), potentially due to the motor paralysis during REM sleep that only develops across infancy. In REM sleep the motor activity happens primarily as twitches. These twitches have

been shown in rodents to be necessary for the motor cortex's development, including mapping of the body (Tiriac et al., 2015). This potentially explains the link with gross motor development. Interestingly, at 12 months, there is a change with *Sleep Activity* being negatively associated with other outcomes. Specifically, *Sleep Activity* at 12 months is linked to infant enterotype with infants in the immature enterotype experiencing more activity during sleep. Additionally, we find that at 12 months *Sleep Activity* is linked to lower scores on gross motor development (however, it is only significant when not including the other sleep variables, but there is a clear switch from positive to negative standardized coefficients from 6 to 12 months). These findings suggest that early on night wakings are normal and a biological process and can potentially affect development positively. However, across the first year, night wakings become less functional, as the stomach capacity should allow for less frequent feedings. Therefore, later in development, night wakings might indicate a sleep problem or less mature sleep behavior. In children and adults, night wakings and sleep fragmentation have been linked to many adverse outcomes such as obesity (van den Berg et al., 2008), lower cortical grey matter volume (Lim et al., 2016), lower school performance (Dewald et al., 2010), and lower health-related quality of life (Magee et al., 2017). It is important to note, however, that the causality of these relationships is unclear.

Another interesting finding concerning *Sleep Activity* is the sex difference we found. Boys had more activity and awakenings during sleep than girls. The findings in the literature thus far were mixed on sex-related sleep differences in infancy, and the studies that found results found it for different aspects of sleep (less daytime sleep in males, longer night wakings in females, So et al., 2007, less sleep quality in males but only at certain ages, A. Scher et al., 2004, longer night wakefulness in males, Bach et al., 2000, no effects Sadeh et al., 2007; Tikotzky et al., 2010). In adults however, the sex difference in sleep quality has been shown several times (Kurina et al., 2015; Roehrs et al., 2006; van den Berg et al., 2009). Our results suggest that these differences are already established in the first year of life and might be linked to biological differences. However, note that there is a sex difference in general activity level in infants (D. W. Campbell & Eaton, 1999), which could potentially explain the results.

Overall we showed that in early life *Sleep Activity* is not linked with adverse bacterial or developmental outcomes and is likely just part of normal development. *Sleep Activity* might only evolve as a marker of reduced sleep quality around 1 year of age or later. In the first few months, parental concerns about night wakings should be put into context of normative sleep data. However, it is also essential to consider the negative effects night wakings might have on the parents. Therefore, if the burden of the night wakings is high in parents, a sleep intervention might be necessary to improve outcomes for parents. Since studies have shown no long-lasting positive or negative impact of these interventions on the child, they can be recommended to parents if they are necessary for parental well-being (Price et al., 2012a).

7.3 HOW TO GIVE EVERYONE THE BEST START IN LIFE?

How does this thesis benefit parents and infants around the world? I would like to point out three ways in which this thesis might contribute to improving healthy infant development.

Firstly, this thesis contributed to developing more standardized and rigorous analysis methods to research infant sleep using actigraphy. Standardized methods improve research because they make results across different studies more comparable, enabling both the development of normative values and replication of results. The improvement of these methods will hopefully benefit future research and lead to higher quality research. Therefore, this will improve our knowledge of infant sleep in the long term.

Secondly, my thesis revealed many interesting aspects of sleep development and the sleep-gut link in infancy. Because our research is based on associations, we cannot conclude any causal relationships. However, our results can build a good basis to develop studies that use experimental interventions such as pre- and probiotics or sleep interventions to influence later sleep or gut outcomes. For example, *Sleep Timing* is easily influenced by parents. Our results hint towards a negative association between late sleep times and later behavioral outcomes. Therefore one potential study could test if, in a group where parents shift the bedtimes to earlier times, infants show higher scores at later behavioral assessments than in a control

group. Similarly, alpha diversity has shown a positive relationship with behavioral outcomes; consequently, intervention studies could increase alpha diversity, e.g., by dietary prebiotics (Ahmadi et al., 2019). Next to intervention studies, our findings should also be translated to research with developmental and health disorders. We proposed *Sleep Day* as a marker for general maturational status. In the next step, studies should investigate if *Sleep Day* is changed in disorders or if early *Sleep Day* is a marker of later disorders.

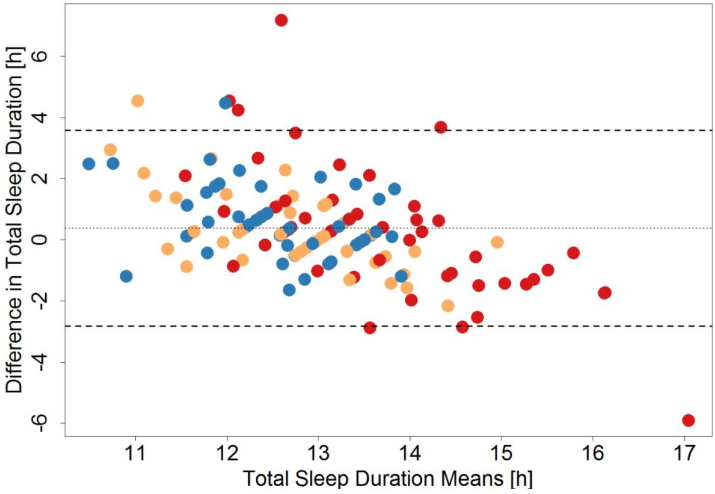
Lastly, my thesis revealed or confirmed infant sleep findings that are directly relevant to parents and practitioners. In this large sample size and with objective data, we confirmed the large variability in sleep behavior both between and within infants that has previously been reported (Iglowstein et al., 2003; Jenni et al., 2007). This variability can, e.g., alleviate parental concerns when they compare their infants to other infants. Additionally, it also shows the transient nature of, e.g., night wakings. Being informed about these transient periods might mitigate some of the stress and adverse mental health outcomes for parents. Additionally, while we found interesting associations between sleep and gut bacteria with developmental outcomes, most associations were small. This suggests that likely only a culmination of risk factors leads to adverse outcomes. Our participants, on average, scored well above the established threshold values for behavioral development scores.

In sum, this thesis provided new insights into sleep across development and showed first evidence of a sleep-gut link in infancy.

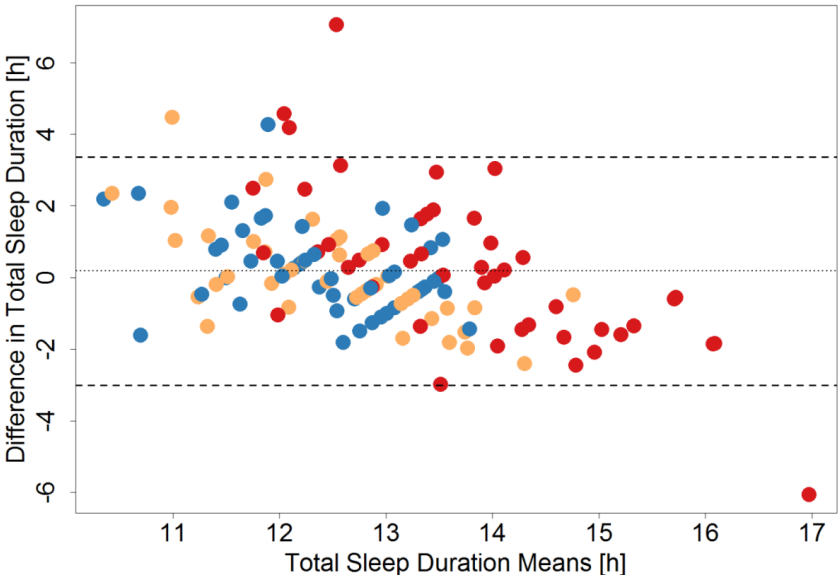
A

APPENDIX

A.1 ARTICLE 2 SUPPLEMENTARY FIGURES



Supplementary Figure 1. Bland-Altman plots of *Total Sleep Duration* estimates from Sadeh algorithm and parental reports (Brief Infant sleep Questionnaire). Each infant is represented by three dots with color indicating age (red = 3 months, orange = 6 months, blue = 12 months). While differences are ~ 0 h in the mean, the critical difference is 3.20 h, indicating large differences between parent reports and objectively measured sleep duration.



Supplementary Figure 2. Bland-Altman plots of *Total Sleep Duration* estimates from Oakley algorithm and parental reports (Brief Infant sleep Questionnaire). Each infant is represented by three dots with color indicating age (red = 3 months, orange = 6 months, blue = 12 months). While differences are ~ 0 h in the mean, the critical difference is 3.19 h, indicating large differences between parent reports and objectively measured sleep duration.

A.2 ARTICLE 2 SUPPLEMENTARY TABLES

***Supplementary Table 1**. Parameters for the models examining agreement between Sadeh and Oakley algorithm. Model 2 that only includes the random effects of the adjustments shows the best model fit.*

	Model 1	**Model 2**	Model 3
Fixed effects	b ± SE [95% CI]	**b ± SE [95% CI]**	b ± SE [95% CI]
Intercept	73.12 ± 0.79 [71.58; 74.66]	**73.12 ± 0.70 [71.76; 74.49]**	73.12 ± 0.76 [71.63; 74.61]
Adjustments	21.39 ± 0.88 [19.66; 23.11]	**21.39 ± 0.92 [19.59; 23.18]**	21.39 ± 0.97 [19.48; 23.29]
Age	4.77 ± 0.35 [4.08; 5.46]	**4.77 ± 0.33 [4.12; 5.41]**	4.77 ± 0.37 [4.03; 5.51]
Adjustments *Age	-3.56 ± 0.45 [-4.44; -2.67]	**-3.56 ± 0.47 [-4.48; -2.65]**	- 3.56 ± 0.52 [-4.58; -2.55]
Random effects	b	**b**	b
Intercept	13.43	**5.02**	5.16
Adjustments	3.59	**3.50**	
Age	1.22		0.42
AIC	1428.3	**1428.6**	1462.9
BIC	1469.0	**1458.2**	1492.5
Pseudo R^2 (marginal; conditional)		**0.89; 0.92**	Model 2

Note: *AIC = Akaike Information Criterion, BIC = Bayes Information Criterion, SE = Standard Error*

***Supplementary Table 2**. Parameters for the models examining agreement between the algorithms and the diary. Model 1 shows the best model fit.*

	Model 1	Model 2	Model 3
Fixed effects	**b ± SE [95% CI]**	b ± SE [95% CI]	b ± SE [95% CI]
Intercept	**68.25 ± 1.20 [65.90; 70.60]**	68.25 ± 0.94 [66.41; 70.10]	68.25 ± 1.21 [65.88; 70.62]
Adjustments	**11.89 ± 0.85 [10.21; 13.56]**	11.89 ± 0.97 [9.98; 13.79]	11.89 ± 0.86 [10.20; 13.57]
Age	**6.57 ± 0.54 [5.51; 7.62]**	6.57 ± 0.43 [5.73; 7.40]	6.57 ± 0.54 [5.50; 7.63]
Algorithm (Sadeh vs Oakley)	**1.58 ± 0.83 [-0.05; 3.21]**	1.58 ± 0.97 [-0.31; 3.47]	1.58 ± 0.86 [-0.10; 3.26]
Adjustments * age	**-1.40 ± 0.42 [-2.3; -0.57]**	-1.40 ± 0.49 [-2.37; -0.44]	-1.40 ± 0.44 [-2.26; -0.56]
Age * Algorithm	**-0.14 ± 0.42 [-0.97; 0.69]**	-0.14 ± 0.49 [-1.10; 0.83]	-0.14 ± 0.44 [-1.00; 0.71]
Adjustments * Algorithm	**-0.97 ± 0.48 [-1.91; -0.02]**	-0.97 ± 0.56 [-2.06; 0.12]	-0.97 ± 0.49 [-1.94; 0.002]
Random effects	**b**	b	b
Intercept	**47.60**	11.24	47.00
Adjustments	**1.90**	0.85	
Age	**7.80**		7.62
AIC	**3218.4**	3304.9	3237.5
BIC	**3280.0**	3353.3	3285.8
Pseudo R^2 (marginal; conditional)	**060; 0.83**		

Note: *AIC = Akaike Information Criterion, BIC = Bayes Information Criterion, SE = Standard Error*

***Supplementary Table 3**. Parameters for the models examining bias between the algorithms. Model 2 that includes only adjustments as random effects shows the best model fit.*

	Model 1	**Model 2**	Model 2
Fixed effects	b ± SE [95% CI]	**b ± SE [95% CI]**	b ± SE [95% CI]
Intercept	351.20 ± 9.88 [331.76; 370.48]	**351.20 ± 9.74 [332.02; 370.22]**	351.20 ± 10.41 [330.72; 371.52]
Adjustments	-363.89 ± 13.31 [- 389.96; -337.81]	**-363.89 ± 13.31 [- 389.97; -337.81]**	-363.89 ± 14.69 [- 392.68; -335.1]
Age	-62.82 ± 4.71 [-72.03; -53.59]	**-62.82 ± 4.70 [-72.03; -53.60]**	-62.82 ± 5.53 [-73.65; -51.98]
Adjustments * age	76.15 ± 6.65 [63.12; 89.19]	**76.15 ± 6.65 [63.11; 89.19]**	76.15 ± 7.82 [60.83; 91.47]
Random effects	b	**b**	b
Intercept	973.98	**839.7**	19.55
Adjustments	1042.97	**1041.6**	
Age	1.55		0.02
AIC	3021.1	**3015.2**	3054.3
BIC	3061.9	**3044.9**	3084.0
Pseudo R^2 (marginal; conditional)		**0.90; 0.93**	

Note: *AIC = Akaike Information Criterion, BIC = Bayes Information Criterion, SE = Standard Error*

***Supplementary Table 4**. Parameters for the models examining bias between the Sadeh algorithm and the diary. Model 3 shows the best model fit.*

	Model 1	Model 2	**Model 3**
Fixed effects	b ± SE [95% CI]	b ± SE [95% CI]	**b ± SE [95% CI]**
Intercept	190.19 ± 21.16 [148.72; 231.66]	190.19 ± 17.99 [154.93; 225.45]	**190.19 ± 20.76 [149.49; 230.89]**
Adjustments	-140.05 ± 21.21 [- 181.62; -98.48]	-140.05 ± 23.81 [- 186.71; -93.39]	**-140.05 ± 21.95 [- 183.06; -97.04]**
Age	-28.79 ± 10.16 [-48.70; -8.88]	-28.79 ± 8.85 [-46.13; -11.45]	**-28.79 ±10.32 [-49.01; -8.57]**
Adjustments * age	17.54 ± 11.09 [-4.19; 39.27]	17.54 ± 12.51 [-6.98; 42.07]	**17.54 ± 11.68 [-5.35; 40.44]**
Random effects	b	b	**b**
Intercept	11532.5	2364.1	**9518**
Adjustments	788.3	694.3	
Age	2086.3		**1910**
AIC	3379.3	3392.5	**3387.8**
BIC	3420.0	3422.1	**3417.4**
Pseudo R^2 (marginal; conditional)			0.37; 0.61

Note: *AIC = Akaike Information Criterion, BIC = Bayes Information Criterion, SE = Standard Error*

***Supplementary Table 5**. Parameters for the models examining bias between the Oakley algorithm and the diary. Model 3 shows the best model fit.*

	Model 1	Model 2	**Model 3**
Fixed effects	b ± SE [95% CI]	b ± SE [95% CI]	**b ± SE [95% CI]**
Intercept	-160.84 ± 20.01 [-200.06; -121.62]	-160.84 ± 16.71 [-193.59; -128.08]	**-160.84 ± 19.77 [-199.58; -122.10]**
Adjustments	223.74 ± 18.51 [187.46; 260.02]	223.74 ± 21.69 [181.23; 266.25]	**223.74 ± 19.16 [186.19; 261.29]**
Age	34.02 ± 9.59 [15.22; -52.81]	34.02 ± 8.07 [18.20; -49.84]	**34.02 ± 9.72 [14.97; 53.06]**
Adjustments * age	-58.59 ± 9.67 [-77.54; -39.64]	-58.59 ± 11.41 [-80.96; -36.22]	**-58.59 ± 10.20 [-78.58; -38.61]**
Random effects	b	b	**b**
Intercept	11769.3	2466	**10354**
Adjustments	630.2	521	
Age	2260.9		**2122**
AIC	3323.8	3323.8	**3333.9**
BIC	3365.6	3365.6	**3363.6**
Pseudo R^2 (marginal; conditional)			**0.44;0.71**

Note: *AIC = Akaike Information Criterion, BIC = Bayes Information Criterion, SE = Standard Error*

A.3 CURRICULUM VITAE

Sarah F. **Schoch**

PhD Student

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Education

PhD in Psychology and Neuroscience — *Zurich, Switzerland*
University of Zurich — *Started in 2016*

- Thesis: "Sleep and Gut Microbiota in Infancy", Supervisor: Dr. sc. nat. Salome Kurth, Steering Committee: Prof. Dr. Moritz Daum, Prof. Dr. Oskar Jenni, Prof. Dr. Reto Huber, Defense planned for Nov 25th 2020
- Brief synopsis: Sleep develops rapidly in the first year of life and infancy is characterized by the highest inter-individual variability in sleep patterns. At the same time the composition and complexity of the bacteria in the human gut undergo large changes. While first studies in animals and adults suggest that there is a bidirectional relationship between sleep and gut microbiota, this has so far not been studied in infants. In my PhD project I am examining the prevalence of sleep behaviors (measured using actigraphy and EEG) in infancy and their association with gut microbial profiles.

Master of Science in Psychology — *Zurich, Switzerland*
University of Zurich — *2014 - 2016*

- Specialization in Cognitive and Neuropsychology
- Master Thesis: "To dream perchance to remember: On the relationship between sleep, dreams and memory" Supervisor: Prof. Dr. Björn Rasch

Bachelor of Science in Psychology, Biology and Education — *Zurich, Switzerland*
University of Zurich — *2010 - 2014*

- Bachelor Thesis: "The Effects of Sleep on Cognitive Functions in Healthy Older Adults: Could a Closer Look at Sleep Improve Cognitive Trainings?", Supervisor: M.Sc. Julia Binder

Experience: Research

Research Assistant and PhD student Project "Sleep and Gut Microbiota in Infancy" — *University Hospital Zurich, Switzerland*
Baby Sleep Laboratory, CRPP Sleep and Health & Clinic for Pulmonology, Dr. Salome Kurth — *July 2016 - present*

- Analyzing sleep EEG data (Matlab)
- Analyzing actigraphy (Matlab and R)
- Analyzing gut microbiota data (R)
- Organizing and conducting longitudinal study with infants

Exchange PhD Student — *Salk Institute, San Diego, USA*
Computational Neurobiology Laboratory, Prof. Dr. Terrence J. Sejnowski — *June 2019 - November 2019*

- Implementing machine learning algorithms (python& tensorflow)
- Downstate detection in brain oscillations (Matlab)

Internship "State Space Analysis in Narcolepsy" — *Unversity Hospital Zurich, Switzerland*
CRPP Sleep and Health, Clinic for Neurology, Dr. med. Lukas Imbach — *02/16 - 06/16*

- Spectral analysis of sleep EEG in Matlab
- State space analysis of Sleep EEG in Matlab

Student Assistant "Approach and Avoidance over the Lifespan" — *University of Zurich, Switzerland*
Developmental Psychology: Adulthood, Prof. Dr. Alexandra Freund — *09/14 - 01/16*

- Preprocessing ECG Data
- Conducting studies with young, middle aged and senior participants
- Creating questionnaires on SoSci Survey

Student Assistant "Hotel Plastisse: iPad Training game for older adults" & "Lebensqualitäts-Barometer UZH" *University of Zurich, Switzerland*

International Aging and Plasticity Center & URPP Dynamics of Healthy Aging, M.Sc. Julia Binder, Prof. Dr. Mike Martin & Dr. Christina Röcke *05/12 - 01/16*

- Conducting and organizing studies including participant contact and support
- Data verification and analysis
- Setting up and testing iPad application and writing a technical documentation
- Planning and implementing large online study on life quality

Student assistant "Neural correlates of trauma memory recall", "Hypnosis and sleep", "Modulation of the Sleep Effect" and others *University of Zurich, Switzerland*

Biopsychology (Sleep and Memory), Prof. Dr. Björn Rasch *10/11 - 01/16*

- Planning and conducting several different studies
- Sleep Scoring
- EEG and MRI acquisition
- Data analysis of behavioural tasks and questionnaires

Student Assistant "Language and Executive Function" & "Inhibition across the Lifespan" *University of Zurich, Switzerland*

Cognitive Psychology, Dr. Miriam Gade & Dr. Alodie Rey-Mermet *10/12 - 11/15*

- Conducting studies including administration of CERAD
- Creation of project websites
- Programming of Simon Task in Tatool with Java

Internship "Modeling of Approach and Avoidance behavior" & "fMRI Analysis of dualTask Paradigm" *University of Florida, USA*

Center for the Study of Emotion and Attention, Prof. Dr. Andreas Keil *06/15 - 09/15*

- Analysis of Behavioural Data in Matlab
- fMRI Analysis with SPM

Student Assistant "Registered Replaction Report for Ego Depletion" *University of Zurich, Switzerland*

Applied Social Psychology, Dr. Johannes Ullrich *01/15 - 06/15*

- Translation of Study Material
- Organization and Conduction of Study

Student Assistant Project Ambizione "Willpower" *University of Zurich, Switzerland*

Motivational Psychology, Dr. Veronika Job *03/11 - 01/15*

- Recruitment and conduction of studies
- Coding of Picturesque Story Excercise (PSE)
- Creating Experiments in Qualtrics (Online Questionnaires) and Inquisit (Stimuli presentation program)

Student assistant "Prospective Memory in older adults" *University of Zurich, Switzerland*

Gerontopsychology, lic.phil. Florentina Mattli *07/11 - 12/11*

- Recruitment of participants
- EEG Acquisition
- Administration of cognitive tests

Experience: Teaching

Co-lecturer of course BME348: Using actigraphy in sleep research *University of Zurich, Switzerland*

Lecturship for Biomedicine at the University of Zurich *11/17*

- Lectures on actigraphy in sleep research
- Supervising student research projects using actigraphy

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Tutorship for lecture "Grundlagen der Entwicklungspsychologie" ("Introduction to developmental psychology") *University of Zurich, Switzerland*

Developmental Psychology: Infancy and Childhood, Prof. Dr. Moritz Daum *09/13 - 06/15*

- Assisting the students
- Managing OLAT (Online learning platform used by the UZH) and "Pearson Maylab and Mastering" (Platform provided by the editor of the book)

Peer-reviewed Publications

Schoch, S.F., Huber, R., Kohler, M., Kurth, S. (2020) Which are the central aspects of infant sleep? The dynamic of sleep composites across infancy. *bioRxiv*, doi: https://doi.org/10.1101/2020.10.26.354803

Gvozdanovic, G.A., **Schoch, S.F.**, Stämpfli, P., Seifritz, E., Rasch, B. (submitted) Neural correlates of sleep-induced benefits on traumatic memory processing

Schoch, S.F., Werner, H. and Kurth, S. (2020). Actigraphy in sleep research with infants and young children: current practices and future benefits of standardized reporting. *Journal of Sleep Research*, https://doi.org/10.1111/jsr.13134

Timofeev, I., **Schoch, S. F.**, LeBourgeois, M. K., Huber, R., Riedner, B. A., and Kurth, S. (2020). Spatio-temporal properties of sleep slow waves and implications for development. *Current Opinion in Physiology*, 15, 172-182, https://doi.org/10.1016/j.cophys.2020.01.007

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Schoch, S.F., Cordi, M.J., Schredl, M., and Rasch, B. (2019). The effect of dream report collection and dream incorporation on memory consolidation during sleep. *Journal of sleep research*, 28(1), https://doi.org/10.1111/jsr.12754

Schoch, S.F., Riedner, B., Deoni, S.C., Huber, R., LeBourgeois, M.K., and Kurth, S. (2018). Across-night dynamics in traveling sleep slow waves throughout childhood. *SLEEP*, 41(11), https://doi.org/10.1093/sleep/zsy165

Schoch, S.F., Werth, E., Poryazova, R., Scammell, T., Baumann, C.R. and Imbach, L.L. (2017). Dysregulation of Sleep Behavioral States in Narcolepsy. *SLEEP*, 40 (12), https://doi.org/10.1093/sleep/zsx170

Schoch, S. F., Cordi, M. J., and Rasch, B. (2017). Modulating influences of memory strength and sensitivity of the retrieval test on the detectability of the sleep consolidation effect. *Neurobiology of Learning and Memory*, 145, 181-189, https://doi.org/10.1016/j.nlm.2017.10.009

Hagger, M. S., Chatzisarantis, N. L., [et al. including **Schoch, S.F.**] (2016). A multilab preregistered replication of the ego-depletion effect. *Perspectives on Psychological Science*, 11, 546–573. https://doi.org/10.1177/1745691616652873

Binder J.C., Zöllig J., Eschen A., Mérillat S., Röcke C., **Schoch S.F.**, Jäncke L. and Martin M. (2015). Multi-domain training in healthy old age: Hotel Plastisse as an iPad-based serious game to systematically compare multi-domain and single-domain training. *Front. Aging. Neurosci.* 7:137. doi: 10.3389/fnagi.2015.00137

Presentations

Schoch S.F., Castro-Mejia J.L., Leng B., Kot W., Krych L., Nielsen D.S., and Kurth S. (2020, October). *From Alpha Diversity to ZZZ: Sleep and the gutmicrobiome in the first year of life* Talk at the Day of Cognition, University of Fribourg, CH

Schoch S.F., Castro-Mejia J.L., Leng B., Kot W., Krych L., Nielsen D.S., and Kurth S. (2020, February). *Developmental associations between sleep and gut microbiome in human infants* Invited talk at the Neuro Meetups in Bern, CH

Schoch S.F. (2020, January). *The role of dreams in memory consolidation during sleep* Invited talk at the Donders CNS lunch meeting, Nijmegen, NL

A

Schoch S.F., Verzhbinsky, I.A., Kim, R., Sejnowski, T.J., and Kurth S. (2019, September). *Improved automatic classification of sleep stages in infants using high-density EEG recordings* Presentation at the World Sleep Congress, Vancouver, CA and at the Salk at Seaside Retreat, La Jolla, US

Schoch S.F., Castro-Mejia J.L., Leng B., Kot W., Krych L., Nielsen D.S., and Kurth S. (2019, June). *Developmental associations between sleep and gut microbiome in human infants* Presentation at the SLEEP Congress, San Antonio, US

Schoch S.F., Castro-Mejia J.L., Leng B., Kot W., Krych L., Nielsen D.S., and Kurth S. (2018, October and 2019, April). *Interplay between sleep, behavioral maturational status and gut bacteria in infant development* Presentation at the International Society for Developmental Psychobiology Congress, San Diego, US, at the Computational Neuroscience Laboratory, Salk Institute, La Jolla, US and at the Swiss Young Sleep Wake Chronobiology Network annual workshop in Gurten, CH

Schoch S.F., Castro-Mejia J.L., Leng B., Kot W., Krych L., Nielsen D.S., and Kurth S. (2018, September). *Tracking infant development: Links between sleep-wake behavior and gut bacteria beta diversity* Presentation at the European Sleep Research Society Congress, Basel, CH and Dataflash at the Annual Meeting of the Swiss Sosciety for Sleep Research, Sleep Medicine and Chronobiology, Basel, CH and as a poster at the annual Neuroscience Center Zurich Symposium in Zurich, CH

Schoch S.F., and Kurth S. (2018, September). *Actimetry in infant sleep research: an approach to streamline algorithms and facilitate comparability* Presentation at the CRPP Sleep and Health Seminar, Zurich, CH

Schoch S.F., Castro-Mejia J.L., Leng B., Kot W., Krych L., Nielsen D.S., and Kurth S. (2018, March). *Relationship between infant sleep behavior and gut bacteria beta diversity* Presentation at the Gordon Research Seminar "Sleep Regulation and Function", Galveston, US

Schoch S.F., Castro-Mejia J.L., Leng B., Kot W., Krych L., Nielsen D.S., and Kurth S. (2018, February). *Relationship between infant sleep behavior and gut microbiome composition* Poster presented at the International Symposium Sleep and Health, Zurich, CH

Schoch S.F., Jenni O.G., Huber R., and Kurth S. (2017, October). *Longitudinal development of macrostructural infant sleep behaviors and relationship with feeding mode and gut microbiota* Presentation at the Child Research Center Retreat, Horgen, Switzerland and ESRS Sleep Science School in Frejus, FR

Schoch S.F., Jenni O.G., Huber R., and Kurth S. (2017, September and October). *Concerns about your baby's sleep: maternal cognitions and infant sleep at 3 and 6 months of age* Poster presented at the World Sleep Congress 2017, Prague, CZ and at the annual Neuroscience Center Zurich Symposium in Zurich, CH

Schoch S.F., Boschert-Hennrich S., Jenni O.G., Huber R., and Kurth S. (2017, August). *Sleep, development and maternal cognition in 3-month-old infants* Poster presented at the Lancaster Conference on Infant and Early Child Development, University of Lancaster, UK

Schoch S.F., Boschert-Hennrich S., Jenni O.G., Huber R., and Kurth S. (2017, May). *Relationship between infant sleep, infant development and maternal cognition in 3-month-olds.* Poster presented at the annual Swiss Society for Sleep Research, Sleep Medicine and Chronobiology meeting in Lugano, Switzerland and at the annual Madoko Congress of the Psychological Institute, University of Zurich, CH

Schoch S.F., and Kurth, S. (2017, April). *The development of sleep and gut microbiota: An Update.* Presentation at the Clinical Research Priority Program "Sleep and Health" Seminar in Zurich, CH

Schoch S.F., and Kurth S. (2017, April). *Quantifying sleep behavior in 3 month old infants.* Presentation at the Swiss Young Sleep Wake Chronobiology Network annual workshop in Kandersteg, CH

Schoch S.F., Riedner B., Dean D.C., O'Muirchearthaigh J., Deoni S.C., Huber R., LeBourgeois M.K., and Kurth S. (2016, September). *Maturational changes in the overnight dynamics of the slow oscillations in the sleep electroencephalogram (EEG).* Poster presented at the annual Neuroscience Center Zurich Symposium in Zurich, CH

Schoch S.F., Schredl M. & Rasch B. (2016, March). *Sleep, Dreams and Memory.* Presentation at the 4th Bern Network Epilepsy Sleep Conciousness Winter Research Meeting in Wengen, CH

Schoch S.F., Schredl M., & Rasch B. (2014, May). *The relationship between dreaming, sleep and memory consolidation.* Poster presented at the LiMaDoKo Congress of the Psychological Institute, University of Zurich, CH

Funding & Grants

2021-2022	**Early Postdoc Mobility Grant** Awarded by the Swiss National Science Foundation	*94'550 CHF*
2018-2020	**Doc.CH Grant** Awarded by the Swiss National Science Foundation	*168'861 CHF*
2019	**Grant for additional travel funds as part of the Doc.CH Grant** Awarded by the Swiss National Science Foundation	*16'610 CHF*
2018	**Grant for Peer-Mentoring Group "Methods and Statistics" (co-applicant)** Awarded by the GRC University of Zurich	*9'980 CHF*
2018	**Travel Grant to attend the ISDP Congress in Sand Diego US** Awarded by the ISDP	*900 USD*
2018	**Travel Grant for a short research stay at the University of Copenhagen, DK laboratory of Prof. Dr. Dennis Nielsen** Awarded by the GRC University of Zurich	*1'000 CHF*
2017	**Grant for the project "Monitoring infant sleep by actigraphy - State of the arte and future" (co-applicant)** Awarded by the Center of Competence Sleep & Health Zurich	*4'000 CHF*
2017	**Grant for Peer-Mentoring Group "Methods and Statistics" (co-applicant)** Awarded by the GRC University of Zurich	*8'990 CHF*
2017	**Travel Grant for the World Sleep Congress in Prague, CZ** Awarded by the SAGW Switzerland	*500 CHF*
2017	**Travel Grant for the 2nd Lancaster Conference on Infant and Early Child Development** Awarded by the Conference organizing committee	*331 CHF*
2017	**Travel Grant to attend the ESRS Sleep Science School in Frejus, FR** Awarded by the Swiss Society of Sleep Research, Sleep Medicine and Chronobiology	*700 CHF*

Awards

2019	**People's choice award Minute-To-Win-It Presentation** Science at the Seaside Salk Congress	*La Jolla, USA*
2017	**Best grant proposal award** Awarded by the ESRS Sleep Science School	*Frejus, France*
2017	**Best presentation award** Awarded by the ESRS Sleep Science School	*Frejus, France*
2017	**1st Place Poster Award** Madoko Congress of the Psychological Institute, University of Zurich	*Zurich, Switzerland*

Professional Service

Ad-hoc Reviewer for

Scientific Reports	*since 2019*
SLEEP	*since 2019*
Journal of Child Psychology and Psychiatry	*since 2019*

Organization

Co-Organizer of "Methods and Statistics" Peer Mentoring group of the Psychological Institute of the University of Zurich, Switzerland	*2017 - 2019*
Co-Organizer of Women in Science, an open discussion round for junior female scientists centered around Zurich, Switzerland	*2016 - 2018*

Outreach

"100 ways of thinking" public event of the University of Zurich participation at ZNZ Day	*2018*

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Presentation for schools "Mehr als ein Bauchgefühl: Die Darm-Hirn Achse" at the BrainFair of the University of Zurich	*2018*
Poster "Die Aktigraphie" presented at the BrainFair of the University of Zurich	*2017*
Participation at "Public Day" of the CRPP "Sleep and Health"	*2015*
Writing Blog entries for the German The Inquisitive Mind Blog http://de.in-mind.org/users/sarah-schoch	*Since 2014*

Mentoring

	PhD Students
Co-Mentoring of Ilya Verzhbinsky, MD-PhD Student	*2019*
	Masterstudents
Co-Mentoring of Lara Barblan, Master Student Psychology	*2018-2019*
Co-Mentoring of Viktoria Gastens, Master Student Pharmacology	*2018*
Co-Mentoring of Rita Grolimund, Master Student Psychology	*2017 - 2018*
Co-Mentoring of Sina Boschert-Hennrich, Master Student Psychology	*2016- 2018*
	Interns
Co-Mentoring of Huijie Liu, Bachelor Student Biology	*2019*
Mentoring of Juliane Berger, Intern Psychology	*2018*
Mentoring of Monika Stoller, Intern Psychology	*2017-2018*
Mentoring of Melanie Auer, Intern Psychology	*2017*

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